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Abstracts

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Skeletal muscle metabolism in exercise and recovery in chronic renal failure. *C. Thompson*, G. Kemp, D. Taylor, P. Barnes, P. Styles, C. Winearls, B. Rajagopalan, G. Radda, MRC NMR Unit, John Radcliffe Hospital, Headley Way, Oxford, OX3 9DU, United Kingdom.* Patients with advanced kidney failure complain of early fatiguability that may be related to anaemia or hyperphosphataemia. The extracellular concentration of inorganic phosphate ($[Pi]_o$) influences intracellular [Pi] ($[Pi]_i$) and $[Pi]_i$ may modify ATP production by altering the phosphorylation potential (P.P. = [ATP]/{[ADP] × [Pi]}) or otherwise influencing mitochondrial function. Using ^{31}P magnetic resonance spectroscopy, we studied skeletal muscle to determine the effects of elevated $[Pi]_o$ on intracellular bioenergetics. 18 non-dialysed patients with chronic renal impairment were studied. The calf muscle was positioned over a surface coil within a superconducting whole body magnet. Spectra were collected at rest, during incremental plantar flexion and recovery, allowing calculation of intracellular pH and [Pi], [phosphocreatine] (PCr), [ATP], [ADP] and the maximum rate of mitochondrial ATP synthesis (Q_{max}). Results were compared with age-matched control subjects. Q_{max} is known to be a sensitive indicator of mitochondrial dysfunction. $[Pi]_i$ was increased in the patient group (Pi/ATP = 0.49 cf 0.38 in controls) and the $[Pi]_i$ increase was correlated with the $[Pi]_o$ ($P < 0.01$). In all patients, [ADP] adapted to the increase in $[Pi]_i$ and hence the P.P. remained constant throughout range of $[Pi]_i$. The constant P.P. suggests no change in the control for oxidative phosphorylation in these uraemic patients. Since metabolism in skeletal muscle at rest is purely aerobic, the constancy of the P.P. implies that P.P. is more important than either [ADP] or $[Pi]_i$ in the control of oxidative phosphorylation. Q_{max} remained constant (35 mM/min) throughout a range of $[Pi]_i$, plasma [creatinine] and [Hb] and was similar to controls (32 mM/min). Constancy of P.P. and Q_{max} over a similar range of $[Pi]_i$ was seen in dialysed patients but, unlike undialysed patients, there were reductions in Q_{max} and P.P. compared to controls. This suggests that control of ATP synthesis was not affected by changes in $[Pi]_i$ and that the institution of dialysis caused metabolic abnormalities in muscle nonexistent in the presence of advanced renal failure prior to dialysis.

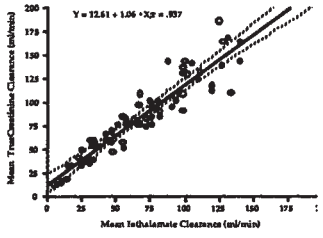
Long-term follow-up of patients with IgA nephropathy: The leading cause of glomerulonephritis in New Zealand. *R.R. Bailey*, K.L. Lynn, R.A. Robson, A.H. Smith, J.E. Wells, Department of Nephrology, Christchurch Hospital, Christchurch.* IgA nephropathy is the commonest form of primary glomerulonephritis in the world. We report the outcome of 151 patients with biopsy-proven IgA nephropathy followed for up to 20 yr. On 30.4.73 the first patient with IgA nephropathy was diagnosed in this department. From then until 21.8.92 a total of 151 patients have had this diagnosis made. Of these, 120 were male giving a male:female ratio of 3.9:1. The age range was 6–74 yr, mean age 32.5, median age 29.6 (SD 15.4) yr. All except 5 were Caucasian. Ninety-five (63%) patients presented with microscopic haematuria, with or without proteinuria and/or renal insufficiency. At presentation renal insufficiency (plasma creatinine ≥ 0.12 mmol/L) was present in 34% and hypertension in 48%. During a mean follow-up period of 63.3 mth (range 0–233 mth) 12 patients have reached end-stage renal failure, 5 have died of a non-renal cause and 25 were lost to follow-up. The actuarial renal survival rate was 92.6% at 5 yr and 91% at both 10 and 15 yr. A significantly poorer prognosis was seen in patients with renal insufficiency, hypertension, 24 hour albumin excretion of ≥1 g, or a serum albumin of <35 g/L at presentation, and this became apparent within the first few years of follow-up. IgA nephropathy has a better outcome than earlier thought. These findings should prove of value in giving an individual patient a fairly precise prognosis at presentation.

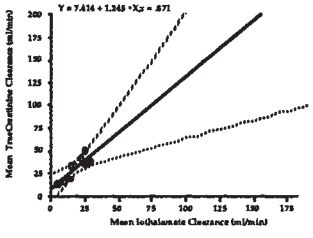
Protein restriction in renal disease: Compliance and consequences. *C.A. Pollock*, L.S. Ibels, R.J. Caterson, D.A. Waugh, J.F. Mahony, Department of Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW, 2065.* Although restriction of dietary protein intake (DPI) is regarded as useful in retarding the progression of renal disease, recent evidence suggests this effect may not be as significant as originally supposed and may result in poor nutritional reserve when end stage renal failure is reached. 680 patients (aged 7–88 years) underwent 24 hour urine collections and the DPI was assessed from urea kinetic modelling. 526 patients had a normal serum creatinine, and of these 360 patients had no dietary restrictions. 166 patients were advised to follow a low DPI. 154 patients had increased serum creatinine of which 105 patients had received advice regarding restricted DPI. 7 patients were assessed before and after commencement of dialysis. Patients with a normal serum creatinine who had no dietary restriction had a significantly higher DPI than those advised in protein restriction (0.79 ± 0.1 vs. 0.72 ± 0.1 g/kg/day (mean ± SEM); $P < 0.0001$). However, in patients with abnormal renal function, dietary protein intake was similar irrespective of dietary advice (0.73 ± 0.03 vs. 0.69 ± 0.02 g/kg/day; $P = 0.28$). A lower DPI correlated with renal dysfunction independent of dietary advice ($P < 0.0001$). In the overall population a low DPI was associated with a lower BMI (24.5 ± 0.3 vs. 25.5 ± 0.2; $P < 0.005$), a lower serum albumin (43.8 ± 0.2 vs. 44.7 ± 0.2; $P < 0.005$), and inverse correlations were observed between DPI and age ($P < 0.0001$), blood glucose ($P < 0.002$), serum cholesterol ($P < 0.002$) and triglycerides ($P < 0.0001$). However, a disproportionate adverse effect on blood glucose and lipids, and serum albumin were not observed in the population with impaired renal function. Patients who reached end stage renal failure had no significant alteration in DPI within a month of commencement of dialysis ($P = 0.86$). The results of the study show that the DPI of the community is lower than expected, but compliance with dietary advice is evident. A low DPI in renal impairment occurs independently of dietary advice. Despite evidence that a proportionately higher carbohydrate intake may have adverse effects on the overall nutritional profile, this is not more common in patients with impaired renal function. Adaptation to a high protein diet following instigation of dialysis is unsuccessful in the short term.

Cardiovascular risk factors are present in children and young adults with renal disease. *L.M. Johnstone*[1], C.L. Jones[1], L.E. Grigg[2], T. Billington[3], J.L. Wilkinson[4], R.G. Walker[1,5], H.R. Powell[1], Departments of Nephrology[1], Cardiology[4], Royal Children's Hospital, Parkville Vic., 3052, Departments of Nephrology[5] and Cardiology[2], Royal Melbourne Hospital, Parkville, Vic., 3050, Department of Chemical Pathology[5], St. Vincents Hospital, Fitzroy, Vic., 3065.* Cardiovascular disease is a major cause of morbidity and mortality in adults with renal disease. Children with end stage renal failure are now being treated successfully with renal replacement therapies, and with long term survival may also be at increased risk of

cardiovascular disease. To evaluate the prevalence of abnormalities in risk factors for cardiovascular disease a cross-sectional study was undertaken in a group of children and young adults with renal impairment or receiving renal replacement therapy. One hundred and thirty-three subjects were enrolled (60 controls, 33 patients with renal impairment (CRF), 10 patients requiring peritoneal dialysis (CAPD) and 30 patients post renal transplantation (TX). Parameters studied included echocardiograph with calculation of left ventricular mass (LVM) and serum lipid (total cholesterol, triglyceride, VLDL-, LDL- and HDL-cholesterols) and lipoprotein (apolipoproteins A1 and B, lipoprotein (a)) concentrations. Serum cholesterol was increased in all groups with renal disease, (controls: 4.6 mmol/l (2.9–7.6), CRF: 5.3 mmol/l (3.2–9.1)**, CAPD: 6.1 mmol/l (3.4–8.2)**, TX: 5.2 mmol/l (3.1–8.1)**); as was VLDL-cholesterol (controls: 0.5 mmol/l (0.2–1.3), CRF: 0.9 mmol/l (0.3–2.0)***, CAPD: 1.2 mmol/l (0.4–16)***, TX: 0.7 mmol/l (0.0–1.5)**). LDL-cholesterol was increased in CRF and TX subjects (controls: 2.6 mmol/l (1.3–5.5), CRF: 3.3 mmol/l (1.1–6.6)***, TX: 3.1 mmol/l (1.5–4.8)**). HDL cholesterol was decreased in the CRF and CAPD groups (controls: 1.44 mmol/l (1.0–3.1), CRF: 1.26 mmol/l (0.44–2.42)**, CAPD: 1.07 mmol/l (0.62–1.28)***). LVM was indexed for height and found to be significantly increased in CAPD subjects (controls: 51.8 g/m (23.1–119.8), CAPD: 80.2 g/m (44.5–100.9)*) and in TX subjects compared to age and sex matched controls (controls 70.2 g/m (25.9–119.8), TX: 97.8 g/m (51.2–182.1)**). These results showed that children and young adults with renal disease have abnormal serum lipids with an atherogenic profile, and have an increased LVM when receiving renal replacement therapy. Thus, children with renal disease would appear to be at greater risk for premature cardiovascular disease. Results expressed as median (range). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Comparison of simultaneous renal clearances of true endogenous creatinine and subcutaneously administered iothalamate in man. *Carol Pollock*, A.Z. Györy, Therese Hawkins, Margaret Ross and Lloyd Ibels, Departments of Medicine, Sydney University and Renal Medicine, Royal North Shore Hospital, St. Leonards, Australia 2065.* Accurate measurement of GFR in clinical practice is still problematic. Endogenous creatinine clearance is the logical method but has suffered from poor reproducibility and accuracy. In 71 (38 females) patients with various renal diseases we compared simultaneously measured ^{125}Iothalamate (IOT) and true endogenous creatinine clearances (TCrCl) over two short collections periods of 1 and 2 hrs. True endogenous creatinine was measured by the Jaffe adsorption method. Range of IOT was 5–141 ml/min. Reproducibility of the IOT method was 18.5% and that of the TCrCl 12.2% as obtained from the two collection periods. Fig. 1 shows the correlation for the whole group, while Fig. 2 for those with IOT less than 30 ml/min. The slope of





the regression was not significantly different from 1 (95% confidence interval (ci) = 0.964–1.155) for the whole group, nor in any subgroup chosen. The intercept at 12.6 ml/min (ci = 5.0–20.2) indicates that there is some creatinine secretion, but that this a constant percentage of TCrCl (unit slope). The probable reason why clearance of creatinine, using methods other than those giving true creatinine values give such widely varying results, is because of variable contribution of other chromogens, and because both serum and urine creatinine change significantly with time if not refrigerated (long collection periods). It is concluded that TCrCl obtained during short collection periods is an excellent, reproducible, accurate and convenient measure of GFR.

Subureteric Polytef injection in the management of vesicoureteric reflux in children. *P.A. Dewan, D.W. Goh*, Urology Unit, Women's & Children's Hospital, Adelaide, SA 5006.* Subureteric Polytef injection is one of the alternatives now available for the management of vesicoureteric reflux in children. The results of the treatment by one surgeon, over a two year period, were studied prospectively in 47 children (60 refluxing units). Following a single injection, reflux was abolished in 49 (82%) ureters and of the 11 failures, 10 ureters showed a reduction of the grade of reflux while one remained unchanged. A repeat injection was performed in nine of these ureters with success in five, persistence in two, and two children are awaiting a follow-up cystogram. The conservative success, with the addition of a second injection, was 90% (54/60). Of the four with unresolved reflux, two have low grade reflux and are free of infection, and two have had a ureteric reimplant, one of which was after a single injection, at the request of the parents. There were no instances of ureteric obstruction. These results show a viable, minimally invasive alternative to open ureteric reimplantation in the management of vesicoureteric reflux in children.

Oral trimethoprim-sulphamethoxazole (TM-SMX) vs. inhaled pentamidine (P) for prevention of pneumocystis pneumonia (PCP) after renal transplantation. *E. Pedagogos*, K. Nicholls, J. Wilson, R. Walker, G. Becker, Depts. Nephrology and Respiratory Medicine, RMH.* 61 patients were randomised to receive prophaxis against PCP with either TM-SMX 800/160 mg B.D. ($N = 32$) or inhalational P 150 mg once a fortnight, commencing 14-days after transplantation and continuing for 6 months. All patients were treated with triple immunosuppression (prednisolone, azathioprine (AZA) and cyclosporin A). 49 patients (26TM-SMX, 23P) completed the study. No cases of PCP were seen. 5 patients died (3TM-SMX, 2P) of non-respiratory causes, 2 further patients (1 in each group) underwent graft nephrectomy for rejection, and 6 patients (2TM-SMX, 4P) were withdrawn. Data was analysed according to intention to treat. The dose of TM-SMX was halved if the serum creatinine level increased >.02 mmol/L ($N = 12$). Both treatment modalities were well tolerated. Cough was common with P, but was controlled by slowing the rate of the inhalation. Renal and respiratory function tests done before treatment, and at 6 months, were not different in the 2 patient groups. White cell counts (WCCs) were not significantly different between groups at any time, but the tolerated AZA doses (decreased from 2 mg/kg/day if WCC <4000/dL) were significantly lower in the P group (median daily doses at 1 month 125 mg TM-SMX, 100 mg P NS: 3 months 141 mg TM-SMX, 75 mg $P < 0.05$; 6 months 117 mg TM-SMX, 61 mg $P < 0.05$. This suggests that P may aggravate bone marrow depression due to AZA (Mann-Whitney U). Our patient numbers are too small to exclude small difference in efficacy between the 2 treatments. We conclude that either TM-SMX or P provides a well-tolerated option for PCP prevention, that the required dose of TM-SMX is probably only 400/80 mg B.D. and that TM-SMX is at least as good as that of P.

Deoxyspergualin inhibits local macrophage proliferation in rat renal allograft rejection. *P.G. Kerr*, D.J. Nikolic-Paterson, H.Y. Lan, S. Rainone, G. Tesch, and R.C. Atkins, Dept. of Nephrology, Monash Medical Centre, Clayton Vic. 3168 Australia.* Deoxyspergualin (DSP) is a potent immunosuppressive drug which can prevent allograft rejection. Although there is good evidence that DSP can inhibit T and B lymphocyte responses, the effect of the drug upon monocyte function is controversial. In the current study, substantial local proliferation of inflammatory macrophages within acutely rejecting rat renal allografts (41.6 ± 5.5% of ED1+ cells on day 5 posttransplantation) was identified by expression of the proliferating cell nuclear antigen (PCNA). This observation was confirmed by bromodeoxyuridine incorporation into macrophages within the tissue. Treatment of animals with deoxyspergualin not only suppressed the degree of mononuclear cell infiltrate, but it also significantly inhibited local proliferation of macrophages within the graft (26.4 ± 5.6% of ED1+ cells, $P <$ 0.05 vs. untreated). This appeared to be, at least in part, a direct effect of DSP upon macrophages since the drug also inhibited growth of two monocytic cell lines (RC-2A, U937) in vitro. However, DSP treatment had no effect upon lipopolysaccharide-induced monocyte IL-1β, TNFα, and IL-6 mRNA and protein production indicating that this drug is not a general inhibitor of monocytes. In conclusion, this study has demonstrated that local proliferation of macrophages within the kidney is a prominent feature of acute allograft rejection and that inhibition of this response is one mechanism whereby DSP exerts its potent immunosuppressive actions.

Prevalence of human T-cell leukemia virus type 1 (HTLV-1) antibodies in Central Australian Aborigines undergoing haemodialysis. *M.G.*

Kirubakaran and L. Mollison, Department of Medicine, Alice Springs Hospital, Alice Springs, NT—0870.* Infection with HTLV-1 is common among Aborigines in Central Australia with reported prevalence rates up to 14%. Dialysis centres in endemic areas of Japan have noted higher prevalence (up to 19.7%) in haemodialysis patients, when compared to healthy blood donors (4.3%). The higher prevalence was unexplained by blood transfusions, but was suggestive of an association between HTLV-1 and chronic renal disease itself. The Central Australian Aborigines have a high incidence of chronic renal failure with unknown aetiology and we examined the sera of patients undergoing haemodialysis for HTLV-1 antibodies, in order to determine its prevalence and its possible association with the underlying renal disease. All patients undergoing haemodialysis at the dialysis unit in Alice Springs had their serum examined for HTLV-1 antibodies after informed consent. The antibodies were detected by a particle agglutination assay and positive results were confirmed with a Western blot at a reference laboratory. Of the 31 Aboriginal patients, 13 (42%) were positive for HTLV-1 antibodies. Their ages ranged from 31–67 years (mean 44.6) and 10 (77%) were males. As a group, the HTLV-1 positive patients did not differ significantly in terms of length of time on dialysis, blood transfusions, age distribution and geographical origin from the HTLV-1 negative patients. However, males were affected more often and all 6 Hepatitis B carriers in the Unit were positive for HTLV-1 also. The aetiology of renal failure was uncertain in 7 out of the 13 (54%) HTLV-1 positive patients, diabetic nephropathy in 2, obstructive uropathy in 2, amyloidosis in 1 and IgA nephropathy in 1. The 6 patients with Hepatitis B antigenemia shared two dialysis machines, but the other 7 did not show any association with the machines used for their haemodialysis. As the antibody status prior to dialysis is unknown in these patients, it is difficult to exclude cross infection as the cause of high prevalence of HTLV-1 among them, but the present data does not support this hypothesis. The strong association with renal failure of uncertain aetiology suggests that HTLV-1 may be related in some way to the renal disease in these patients. In view of the high incidence and possible aetiological association, routine screening for HTLV-1 is recommended for all Aboriginal patients prior to entry into dialysis programs.

Discontinuous use of stored BiCart dialysis concentrate does not constitute a microbiological hazard. *D.W. Johnson*, J. Dale, N. George, J. Faoagali, S.J. Fleming, Departments of Renal Medicine and Microbiology, Royal Brisbane Hospital, Q1d, 4029.* To assess the potential bacteriological risks associated with discontinuous use of stored *BiCart* cartridges, total viable microbial counts were performed on 30 specimens of water used to prepare dialysate, 20 unused *BiCart* cartridges, 105 *BiCart* cartridges stored wet for 48 hours following single use and 21 samples of dialysate derived from these stored cartridges. The median bacterial counts were 89 colony forming units (CFU)/ml, 0 CFU/ml, 0 CFU/ml and <25 CFU/ml, respectively. Corresponding cultures of liquid bicarbonate concentrate before use ($N = 75$) and 48 h later ($N = 75$) together with 20 dialysate samples derived from the stored concentrates, yielded median counts of 0 CFU/ml, 0 CFU/ml and 200 CFU/ml, respectively. The most common organisms grown from all forms of dialysate or concentrate were pseudomonads. *BiCart*-derived dialysates universally complied with the standard of 2000 CFU/ml set by the Association for the Advancement of Medical Instrumentation (AAMI). These results indicate that discontinuous use of *BiCart* cartridges after storage for up to 48 hours results in relatively low levels of bacterial contamination of dialysate, and could result in significant cost reductions without compromising patient safety.

The role of parvovirus infection in mediating bone marrow suppression following renal transplantation. *N. Hay*, C. Pollock, T. Chang-Zheng, D. Morgan, J.F. Mahony, R.J. Caterson, D.A. Waugh, L.S. Ibels, Y. Cossart, Dept. Renal Medicine, Royal North Shore Hospital, St. Leonards NSW 2065 and Dept. Infectious Diseases, University of Sydney, NSW 2006.* Human parvovirus B19 causes a wide spectrum of diseases ranging from Fifth Disease in children to aplastic anaemia, thrombocytopenia, arthritis and fetal hydrops. The virus predilection for rapidly dividing cells and its tissue tropism determined by the presence of the viral receptor (the P blood group antigen) explain these diverse manifestations of infection. Very prolonged anaemia due to persistent B19 infection has been described in immune deficient patients, and this has been treated successfully with normal pooled immunoglobulin. Recently it has been found that HIV positive patients with thrombocytopenia have a high prevalence of B19 IgM antibody. Unexpectedly, these patients may become viraemic for B19 despite prior evidence of recovery from infection (B19 IgG). It has been postulated that the replication of HIV may be able to provide permissive conditions for B19 in the bone marrow, and that the viraemia may represent reactivation of latent B19 DNA. Renal transplant patients also suffer from refractory anaemia and thrombocytopenia and are immunosuppressed. In addition they may have received prior erythropoietin which could stimulate red cell precursor turnover and allow reactivation of B19. To seek evidence of B19 reactivation in another clinically significant setting we have performed B19 IgM and IgG serology on pre- and post-transplant sera from 36 patients. 24 were B19 IgG positive pre-transplant which is comparable with the rate in blood donors. B19 IgM was present in 9 post transplant sera and 6 of these were IgG positive earlier. 2 other patients were viraemic as judged by B19 PCR. B19 sequences are being sought in bone marrow aspirates from patients with and without serological evidence of virus activation or clinical bone marrow suppression.

The comparative efficacy of 3 regimes of i.v. iron dextran in haemodialysis (HD) patients treated with erythropoietin (EPO). *D. Saltissi*, D. Sauvage, J. Westhuyzen, Department of Renal Medicine, Royal Brisbane and Keperra Hospitals, Brisbane, Queensland.* Uraemic anaemia is primarily due to relative EPO deficiency. The demand for new red cells is most marked in HD patients due to constant blood, thus iron losses. rHuEPO administered parenterally alleviates uraemic anaemia, but the response is blunted by several factors including most commonly, iron deficiency. In many cases parenteral iron has been shown necessary to restore EPO efficacy, but the optimum time course of administration is unknown. We have performed an open randomised study of I.V. Iron Dextran (Imferon*) in 60 deficient patients (ferritin <100 μg/L and/or transferrin saturation <20%). Elemental iron was administered either as a single 600 mg bolus (A), or 100 mg weekly for 6 weeks (B), or 100 mg on 6 successive dialyses (C) (20 patients each). Haematological response was monitored for eight weeks. Statistical analysis indicates all 3 methods have an effect on iron parameters, with the upward trend of Hb reaching significance ($P < 0.01$). As there were no differences between the methods, single dose infusion (method A) would seem most expedient. * Registered Trademark, Fisons Pty Ltd.

Clinical and laboratory assessment of two dialyser variables: sterilisation process and porosity. *P.G. Kerr*, A.M. Corradini, O. McGee, K.C. Wong, R.C. Atkins, Dept. of Nephrology, Monash Medical Centre, Clayton, Vic.* There are many potential dialyser variables which may influence patient well-being and tolerance of dialysis. In an attempt to isolate two of these factors we utilized cuprammonium dialysers in three different conformations, 5 times each in 5 patients. The "baseline" was ethylene oxide (ETO) sterilised, standard cuprammonium membranes. The variables included were a change to steam sterilisation (SS) and then the addition of "high-flux" cuprammonium membranes (HF) which were also steam sterilised. The clinical variables measured for all dialyses were BP (pre and post dialysis), weight loss achieved, dialysis related symptoms, and temperature rise. During the 5th dialysis instantaneous dialyser urea clearance (measured 1 hour into dialysis at 200 ml/min blood flow), and β_2 microglobulin clearance (corrected for protein concentration, expressed as percent reduction) were measured. Plasma was also obtained for pre and post dialysis IL-1β and TNFα assessment (by ELISA). There were no differences in BP or temperature rise between the 3 groups. There were more symptoms with the SS group compared to the other 2 groups (though not significant, $P = 0.1$). Instantaneous dialyser clearances were significantly better with the HF dialysers (174 ± 6 ml/min with ETO, 178 ± 2 with SS, and 184 ± 1 with HF; $P < 0.05$ HF vs. ETO or SS). β_2m clearances were negligible with ETO and SS dialysers but were 18.6 ± 3.5% with the HF group ($P < 0.05$ vs. ETO or SS). IL-1β and TNFα plasma levels were mildly elevated predialysis, did not rise with dialysis, and showed no difference between the groups. Thus steam sterilisation alone offered no benefit in this group of patients and the high-flux version of cuprammonium dialysers offered significantly improved urea clearances but still only fair β_2m clearance. Cytokines were not significantly induced over the course of dialysis by any of the membranes. Choice of these more expensive membrane variables cannot be recommended on biocompatibility grounds.

Sclerosing peritonitis—Identification of diagnostic, clinical and radiological features. *S. Campbell*, C. Hawley, P. Clarke, M. Wigan, P. Kerlin,*

J. Butler and D. Wall, Departments of Renal Medicine, Radiology, Gastroenterology and Surgery, Princess Alexandra Hospital, Woolloongabba, Brisbane, 4102. Sclerosing peritonitis is a rare, but serious complication of peritoneal dialysis. In an attempt to identify patients at risk of developing this life-threatening problem early, we performed a cross-sectional study of 15 patients. Five had died of sclerosing peritonitis. Four had stopped peritoneal dialysis because sclerosing peritonitis was suspected. Six were considered to be at increased risk because of more than four years on peritoneal dialysis. We looked at duration of dialysis, number of episodes of peritonitis, strength of bags, as well as type of dialysate and use of beta-blockers. We also used a number of radiological investigations, including abdominal x-ray, a measure of colonic transit using radio-opaque markers, abdominal ultrasound and CT-scan. Of the clinical features, only duration of dialysis could be shown to be an important risk factor. We identified a number of radiological features which we believe to be early signs of peritoneal sclerosis. The two CT-scans that were available from deceased patients, demonstrated peritoneal thickening, as did those in three of the four living patients who stopped peritoneal dialysis with suspected disease, and two of the six patients on peritoneal dialysis for over 4 years. Ultrasound demonstrated a characteristic trilaminar appearance in four patients, but was unable to be demonstrated without peritoneal fluid in situ. Delayed colon transit was demonstrated in three of the four living patients with clinically suspected disease. Radiological screening to detect sclerosing peritonitis early in high risk patients, requires further study.

Anti-N_{form} antibody in haemodialysis patients. *Y-Y. Ng*, M-P. Chow, D.C.H. Harris and T-P. Huang, Depts. of Renal Medicine, Veterans General Hospital, Taipei, Taiwan and Westmead Hospital, NSW 2145.* The anti-N_{form} antibody is produced by dialysis patients following reuse of dialysers sterilised with formaldehyde and it has been implicated as a cause of haemolytic anemia. Formaldehyde is one of the common disinfectants used for reprocessing capillary haemodialysers. The safety of formaldehyde and the clinical significance of anti-N_{form} antibody need further evaluation. Amongst 45 patients practising dialyser reuse, anti-N_{form} antibody was detected in 5 (11.1%), but not amongst 111 patients not reusing their dialyser ($P < 0.005$). The presence of anti-N_{form} was not related to duration of dialysis or duration of dialysis with reused dialysers. There was no overt haemolysis in the patients with positive anti-N_{form} antibody, although significant haemolysis occurred in one patient prior to this study. Direct Coomb's test was positive in 80% of all tested patients with anti-N_{form} antibody, and in 38% of patients reusing dialysers but without anti-N_{form} antibody. No test of haemolysis (including direct Coomb's test) discriminated between anti-N_{form} antibody positive and negative patients, nor between anti-N_{form} antibody patients with and without overt haemolysis. The best diagnostic test for haemolysis in anti-N_{form} antibody positive patients is haematocrit rise after cessation of dialyser reuse.

Evaluation of the UV-Flash germicidal exchange device for use in peritoneal dialysis (PD). *D. Saltissi*, M.J. King, Department of Renal Medicine, Royal Brisbane and Keperra Hospitals, Brisbane, Queensland.* Numerous techniques have been devised in attempts to reduce peritonitis incidence with chronic PD. The UV-Flash (Baxter*) is a recent development combining automated spike-bag connection with Xenon Ultraviolet sterilisation. In a 12 mth prospective randomised cross-over study we have investigated peritonitis incidence and patient acceptability of the UV-Flash compared to "other" systems. 24 new pts (13 M, 11 F; age 27–80, mean 57 yrs) were randomly allocated UV-Flash (12 pts) or "other" system (Ultraset 4, System III 4, CXD 4) and crossed after six months.

Results:		UV-Flash	"Other" System
Episodes Peritonitis	1st 6m	10 (10pts)	4 (4pts)
	2nd 6m	0 (5pts)	2 (pts)
Overall Peritonitis rate		1:9.96 mth	1:14 mth
		P = NS	

10 pts did not complete the two study periods, reasons will be discussed. Numerous technical problems were initially encountered, all were eventually rectified. Elderly patients tended to fear the device. Cost per annum: UV-Flash, $14,700, System III $14,000, Ultraset $20,000. We believe the UV-Flash has a place particularly for patients with visual and manual dexterity problems.

Low molecular weight heparin use in haemodialysis: A comparison with standard heparin and a cost-analysis. *Nicola M. Hay* and Robyn J. Caterson, Department of Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW 2065, Australia.* A stable patient group receiving treatment at a satellite dialysis unit was assessed with regard to anticoagulant use during haemodialysis. Heparin is the standard anticoagulant used to prevent clotting in the extracorporeal circuit, given as a bolus injection at the start of dialysis followed by a continuous infusion. Low molecular weight heparin (Fragmin^R) has a longer half-life allowing for more convenient administration as a single injection given at the start of dialysis only. No monitoring of clotting factors is required with either regimen. Twenty-seven patients (11 males) with a mean age of 51 years (range 25 to 77) were assessed over a three month period. In the group receiving conventional heparin ($N = 18$) the cost was calculated as $1.77 per dialysis treatment. The mean "compression time" was 9.14 minutes (range 2.5–16.25). Nine patients received Fragmin^R, 6 receiving 2500 anti-Xa units ($3.80) and 3 requiring 5000 anti-Xa units ($7.58). The mean "compression time" was 8.62 minutes (range 3.8–13.3). There were no bleeding complications with either treatment. When a patient requires minimal anticoagulation monitoring of whole blood clotting times is required if low dose heparin is given. This results in a significantly greater cost of $10.92 compared to treatment with 1250 anti-Xa units of Fragmin^R ($3.80). Our observations suggest that it is clearly not cost-effective to use Fragmin^R during routine haemodialysis, however, there is a cost advantage when minimal anticoagulation is required. Fragmin^R is a safe, convenient, and effective but expensive alternative to standard heparin used during haemodialysis.

Effect of enalapril on haemorheology in hypertensive patients with renal disease. *B.I. Shand,* R.R. Bailey, K.L. Lynn and R.A. Robson, Department of Nephrology, Christchurch Hospital, Christchurch.* We assessed prospectively, whether the angiotensin converting enzyme inhibitor (ACEI), enalapril, caused changes in haemorheology (blood viscosity) in hypertensive patients with renal disease. Cellular and plasma determinants of blood viscosity, renal function and blood pressure were tested at baseline and 2, 60 and 120 days following treatment with enalapril (mean dose 5.4; range 2.5–20 mg/day) in 19 patients with a range of renal disorders with complicating hypertension. None of the patients was taking other antihypertensive medication or diuretics during the study and all other therapy remained unchanged. Approximately one-half of the patients had an abnormal baseline blood rheology profile. Within 2 days following enalapril treatment there was a significant decrease in blood viscosity (−0.15 mPa.s; $P < 0.05$), followed by a further fall of similar magnitude by day 60, which was unchanged by day 120. The initial decrease in blood viscosity correlated significantly with a concurrent fall in mean plasma viscosity ($r = 0.80$; $P = 0.001$) and plasma albumin concentration ($r = 0.49$; $P = 0.01$), indicating haemodilution had occurred secondary to ACEI-induced vasodilatation. The later further reduction in blood viscosity was mainly associated ($r = 0.82$; $P = 0.001$) with a small but significant fall in haematocrit (−0.02; $P = 0.01$) and also to a trend of improved red blood cell and white blood cell rheological properties. We conclude, that enalapril has a beneficial effect on haemorheology in patients with renal disorders. This effect is related, initially to intravascular volume changes and subsequently, to a small decrease in haematocrit and improved blood cell rheological properties. The decrease in blood viscosity would be expected to reduce vascular resistance to blood flow and therefore is complementary to the antihypertensive action of enalapril.

Effect of enalapril on haematological indices and plasma erythropoietin in hypertensive patients with renal disease. *B.I. Shand,* R.R. Bailey, K.L. Lynn and R.A. Robson, Department of Nephrology, Christchurch Hospital, Christchurch.* We have shown previously that angiotensin converting enzyme inhibitors (ACEIs) cause small reductions in haematocrit in healthy subjects, associated with a decrease in plasma erythropoietin (EPO) concentration. We studied this effect prospectively in 19 hypertensive patients (mean age 47 ± 11 yr; 14 males) with a range of renal diseases who were treated with enalapril. Blood samples were obtained at baseline and at 2, 60 and 120 days following administration of enalapril (mean dose 5.4; range 2.5–20 mg/day) and tested for haematological

indices, plasma EPO (measured in duplicate by radioimmunoassay) and plasma creatinine concentrations. By day 60 there was a decrease in haemoglobin concentration (mean decrease 7.4 g/l; $P = 0.03$) that was sustained until day 120. The magnitude of the decrease in haemoglobin concentration correlated significantly with the baseline plasma creatinine concentration ($r = -0.606$; $P < 0.01$). A decrease in plasma EPO concentration occurred by day 2 (mean decrease 16.8%; $P = 0.04$) followed by a return to baseline. The changes in plasma EPO concentration of individual patients, were not related to either the dose of enalapril, baseline haematological status or renal function, or to the subsequent decrease in haematocrit. These results suggested that the degree of renal impairment was important in determining the magnitude of the decrease in erythropoiesis associated with ACE inhibition. The mechanism of these haematological changes however remains unclear. Our findings are consistent with other studies that have demonstrated that angiotensin II is necessary for the maximal production and erythropoietic action of EPO. Alternatively, an as yet unidentified factor involved in the control of erythropoiesis may be affected by ACE inhibition.

Tubular silicon accumulation in chronic renal disease (CRD). *B.J. Nankivell*, R.A. Boadle, M. Akram, D.C.H. Harris, Dept. of Renal Medicine & EM unit, Westmead Hospital, Sydney.* Epidemiological evidence suggests that silicon is nephrotoxic. As silicon is primarily excreted by the kidney as silicic acid, it is possible that silicon may be involved in the progression of CRD. The aim of this study was to determine the renal disposition of silicon in experimental and human CRD. Tubular silicon was assessed by energy dispersive X-ray microanalysis and quantified against a biological standard in several models of CRD in Wistar rats, including remnant kidney (RK), puromycin nephrosis, Adriamycin nephrosis and streptozotocin diabetes. Substantial amounts of silicon were seen in uraemic RK ($P < 0.01$ vs. sham), and modest amounts in the proteinuric, non-uraemic models of renal disease at 8 weeks. In RK silicon was observed predominantly in proximal tubular secondary lysosomes and tubular cytoplasm ($P < 0.01$ vs. background), often associated with aluminium. Silicon was seen as early as four weeks and reached a plateau after that. In RK after six months, silicon accumulation correlated with impairment of Tc^{99m} GFR ($r = 0.91$). Tubular damage correlated with the number of silicon-containing lysosomes ($r = 0.80$, $P < 0.05$). Preliminary examination of human renal biopsies also demonstrated tubular silicon accumulation in CRD ($P < 0.001$ vs. normal). These data suggest silicon accumulation is independent of proteinuria, it occurs as an aluminosilicate, and may be due to the increased load to the remaining nephrons. Although the relationship of silicon to impairment of GFR and tubular damage is suggestive a role in progressive CRD, further research is required to determine a causal relationship.

Low protein diet is not protective in the remnant kidney model. **A.M. Walker, N.M. Thomson, Dept. Medicine, Monash Medical School, Alfred Hospital, Prahran, Melbourne, Vic., Australia, 3181.* Low protein diet has been demonstrated to inhibit compensatory renal growth (CRG) and slow the development of renal failure and glomerulosclerosis in rat remnant kidney model of chronic nonimmune injury. 12 week old, male inbred SD underwent either 7/8 nephrectomy by infarction (Nx) or sham laparotomy (Sh). Rats were randomly assigned to receive standard protein diet (25% protein) (St) or low protein (8%), isocaloric diet (LP). Growth, blood pressure and renal function was followed for 12 postoperative weeks. Systolic blood pressure was measured by tail-cuff method and renal function estimated by sCr, Cr. clearance and urinary protein excretion. Animals were killed at regular intervals over a 12 week period and tissue assessed for histological damage. Low protein diet following Nx was associated with somatic growth failure (332 ± 9 g LPNx, 366 ± 8 g Nx at 9 w) and the early development of severe hypertension (200 mm Hg LPNx, 150 mm Hg StNx, 120 mm Hg LPSh & StSh) by 4 w. Renal function was similar for the first 4 weeks but then deteriorated more rapidly in those animals receiving the LP diet: sCr 180 ± 75 mmol/l (LPNx), sCr 118 ± 21 mmol/l (StNx) at 8 weeks. Most animals receiving LP diet were in end stage renal failure by 11 w. Histological analysis revealed similar degrees of glomerular hypertrophy between the dietary groups (47 ± 2% glom LPNx, 50 ± 5% glom. StNx at 9 weeks). Percent glomeruli demonstrating sclerosis was less in the LPNx group despite worse renal function when compared to StNx dietary group (11 ± 2% LPNx, 21 ± 6% StNx at 9 w). Although there appeared to be more severe tubular damage and dilatation in LP diet animals, tubulointerstitial index was similar between the groups. It is unclear why dietary modification with low protein, isocaloric feed would lead to such severe hypertension and renal deterioration following 7/8 Nx.

Cancers of the renal pelvis are more malignant when associated with phenacetin-containing analgesics: Evidence for a carcinogenetic-promoting effect of papillary necrosis. *J.H. Stewart*, J.B. Hobbs and Margaret McCredie, University of Sydney, Nepean Hospital and NSW Cancer Council, PO Box 63, Penrith, NSW, 2751.* The histopathological slides of 101 kidneys with pelvic cancer, drawn from a population-based sample of 147 respondents to a questionnaire eliciting information concerning exposure to risk factors for kidney cancer, were reviewed 'blind' to the exposure status of the cases. Papillary necrosis (odds ratio—OR—6.3), papillary calcification (OR 3.7) and diffuse papillary scarring (OR 4.4) were significantly ($P < 0.02$) associated with heavy consumption (>1 kg) of phenacetin-containing analgesics, but focal papillary scarring and ureteric submucosal capillarosclerosis were not. The overall histological (ASH) and nuclear grades of the transitional-cell carcinomas (TCC) were significantly ($P < 0.02$) higher, but there was no increase in squamous metaplasia nor squamous-cell carcinoma, in analgesic-associated cancers. Neither the histological grade of cancer nor the degree of squamous change was related to smoking tobacco. Multivariate logistic regression identified female sex (OR 4.8; possibly representing confounding factors), papillary necrosis (OR 4.7) and diffuse papillary scarring (OR 5.7), but not heavy analgesic consumption, as independent factors significantly associated with a higher grade of malignancy. These observations support the concept that an important part of the carcinogenetic effect of heavy consumption of phenacetin-containing analgesics is promotion by the papillary damage so caused.

Pregnancy in renal transplant recipients—The Christchurch experience. *K.M. Wong*, R.R. Bailey, K.L. Lynn, R.A. Robson, Department of Nephrology, Christchurch Hospital, Christchurch.* Between July 1972 and 31 December 1992 74 women in the 15–45 yr age group had a total of 93 transplants in this institution. During this time 16 pregnancies were recorded in 9 of these women. Fourteen of the pregnancies were planned. These resulted in 11 live births and 3 first trimester abortions. The remaining 2 were unplanned and were terminated. Mean age at transplantation of these 9 women was 17.2 [SD 6.8; range 16–22.5] yr and mean interval from transplant to pregnancy was 6.0 [SD 3.7; range 0.2–9.0] yr. Thirteen pregnancies occurred in 7 women with a cadaveric kidney and 3 pregnancies in 2 women who had received a kidney from a living-related donor. Prednisone and azathioprine were used in all patients and cyclosporin in 5 of the pregnancies. For 7 of the successful pregnancies the pre-conception plasma creatinine was ≤0.10 mmol/l and was unchanged after delivery. One of these women developed chronic rejection 5 yr after delivery and reached endstage renal failure 9 yr later. For the other 4 successful pregnancies the pre-conception plasma creatinine was 0.12 to 0.14 mmol/l. One of these patients halved her renal function, but this has remained stable for 15 yr, 1 poorly compliant woman developed chronic rejection after delivery and reached endstage renal failure 2 yr later, 1 developed cyclosporin toxicity and the other was unchanged. Six deliveries were by elective caesarean section, 4 by urgent caesarean section and 1 by vaginal delivery. There were no congenital anomalies or perinatal mortality. One infant died aged 4 mth from a cot death. We recommend that if a woman has had a stable renal transplant for >2 yr, graft function is normal, and BP well controlled then there is no contraindication to her having a pregnancy which should be managed by shared obstetrical/nephrological care.

Cutaneous manifestations of renal transplantation in a New Zealand population. *D.J. Hepburn, D. Divakar, R.R. Bailey*, K.J.S. Macdonald, Departments of Dermatology and Nephrology, Christchurch Hospital, Christchurch.* Long-term immunosuppressive therapy is essential following organ transplantation. This increases the rate of cutaneous infection and malignancy. We have documented our experience with 52 stable renal transplant recipients (27 males; mean age 43.5 [SD 12.0] yr; mean duration of immunosuppression 115.6 [SD 70.6] mth; range 3–258 mth) from a temperate maritime climate, latitude 42°S, with regard to sun exposure, skin type and human papillomavirus (HPV) type of clinically apparent warts. All skin infections were characterised and miscellaneous cutaneous lesions recorded. Thirty-seven patients were on a regimen of prednisone and azathioprine and the remaining 18 on prednisone, azathioprine and cyclosporin. Forty-three (84%) were regular users of sunblock with a sun

protection factor rating of ≥15. Premalignant and malignant lesions were common (20 actinic keratoses, 3 BCC, 2 KA, 1 melanoma). Including prior observations 48 cutaneous malignancies were identified in 9 patients. Squamous cell carcinoma outnumbered basal cell carcinoma by a ratio of 1.2:1. No metastases were recorded. Non-neoplastic lesions were also documented including the prevalence of HPV infection. There was a significant correlation between the development of skin cancer after transplantation and total sun exposure ($P < 0.05$), clinically apparent wart infection ($P < 0.05$), and the duration of immunosuppression ($P < 0.01$). Renal transplant recipients require regular skin examination. High risk patients for skin cancer should be identified as they require intensive dermatological follow up.

Mechanisms of glomerular neutrophil recruitment in a complement independent model of glomerulonephritis. *P.G. Tipping, X.R. Huang, S.R. Holdsworth, Monash University, Dept. of Medicine, Monash Medical Centre, Clayton 3168.* Neutrophil recruitment and lung injury following complement activation has been demonstrated to be dependent on endothelial expression of P selectin. In anti-glomerular basement membrane antibody induced glomerulonephritis (anti-GBM GN) in mice, acute glomerular injury results from complement independent, neutrophil accumulation. The signals for neutrophil recruitment in this model are unknown. Expression of P selectin on glomerular endothelium was demonstrated within 30 minutes of administration of anti-GBM antibody to C57/BL10 mice. This was associated with rapid accumulation of neutrophils in glomeruli which peaked at 1 hour (6.2 ± 0.5 neutrophils per glomerular cross section [neut/gcs], normal 0.34 ± 0.06 neut/gcs, $P < 0.01$) and significant proteinuria after 16 hours (3.6 ± 0.5 mg/16h, control 0.62 ± 0.13 mg/16h, $P < 0.01$). Complement depletion with cobra venom factor, which reduced serum C3 levels to less than 5% of normal, did not alter expression of P selectin, reduce glomerular neutrophil accumulation (6.7 ± 0.8 neut/gcs) or proteinuria (3.7 ± 0.5 mg/16h). Platelet depletion also failed to alter glomerular expression of P selectin, neutrophil accumulation or the development of proteinuria. Mice were treated with an affinity purified anti-human P selectin antibody, which crossreacted with mouse P selectin and blocked P selectin dependent binding of thrombin activated mouse platelets to HL60 cells. Treatment, 1 hour prior to administration of anti-GBM antibody, markedly inhibited glomerular neutrophil accumulation (0.94 ± 0.12 neut/gcs) and prevented proteinuria (1.0 ± 0.2 mg/16h), and did not alter binding of anti-GBM globulin in the kidney. These data strongly suggest that the rapid upregulation of P selectin expression in glomeruli following binding of anti-GBM antibody is an essential signal for neutrophil recruitment in this complement independent model of glomerular injury.

Suppression of established rat crescentic glomerulonephritis (GN) by the interleukin-1 receptor antagonist (IL-1ra). *H.Y. Lan, D.J. Nikolic-Paterson, W. Mu, R.C. Atkins, Dept. of Nephrology, Monash Medical Centre, Clayton Vic 3168, Australia.* We have previously demonstrated that IL-1ra treatment from the time of anti-GBM serum injection suppressed the development of accelerated rat anti-GBM GN. The current study investigated whether IL-1ra treatment could intervene in established crescentic GN. Accelerated anti-GBM GN was induced in inbred Sprague-Dawley rats by priming with 5 mg normal rabbit IgG and injection of rabbit anti-rat GBM serum 5 days later (day 0). One group of 6 rats received a constant subcutaneous infusion of IL-1ra from day 7 until being killed at day 21. Control animals have the same experimental schedule but received saline only (untreated). Blood and urine samples were taken on days 0, 1, 7, 14, and 21. Leukocyte infiltration was assessed by immunoperoxidase staining with monoclonal antibodies. Saline treated animals showed marked disease progression over days 7 to 21 which was significantly reduced by IL-1ra treatment. Specifically, IL-1ra treatment reduced hematuria ($P < 0.001$), proteinuria ($P < 0.05$), and plasma creatinine while improving creatinine clearance ($P < 0.05$, by ANOVA). Histologically, IL-1ra treatment markedly reduced glomerular hypercellularity and segmental sclerosis ($\downarrow 50\%$, $P < 0.01$), cell proliferation ($\downarrow 53\%$ of PCNA cells/gcs, $P < 0.001$), glomerular crescents (58.5 ± 4.9% vs. 25 ± 3.6%, $P < 0.01$), and tubulointerstitial fibrosis ($P < 0.05$). Immunohistologically, IL-1ra treatment suppressed glomerular ($\downarrow 42\%$, $P < 0.01$) and interstitial ($\downarrow 64\%$, $P < 0.001$) macrophage accumulation and interstitial T cell accumulation ($\downarrow 37\%$, $P < 0.01$). Importantly, IL-1ra suppressed interstitial mononuclear cell activation ($\downarrow 64\%$ IL-2R cells, $P < 0.01$). This study demonstrates that IL-1ra can intervene in progressive renal injury in experimental crescentic GN.

Reactive oxygen species (ROS) and lipid peroxidation (LPO) in proteinuric experimental renal disease. *T.J. Neale*, D. Kerjaschki#, J. Witztum†, P. Davis, B. Rüger, Dept. of Medicine, Wellington School of Medicine, #Institute of Pathology, University of Vienna and †Department of Medicine, UCSD, San Diego.* ROS are implicated in the pathogenesis of experimental glomerular injury. In proteinuric passive Heymann's nephritis (PHN), glomerular epithelial cells (GEC) show heightened expression of cytochrome b558, a component of the respiratory burst oxidoreductase that produces ROS (*Proc Natl Acad Sci USA* 90:3645–3649, 1993). Cytochrome b558 also showed increased expression in the GEC of amminonucleoside nephrosis (AN) rats, by monoclonal IF microscopy and immunoblotting of isolated glomerular extracts. There was marked upregulation of cytochrome mRNA by Northern blotting. H_2O_2 was localised by EM within the glomerular capillary wall using *ex vivo* perfusion with cerium. In proteinuric AN and PHN glomeruli malondialdehyde (MDA) was revealed as a marker of LPO with antibody specific for MDA-lysine adducts by IF microscopy, immunogold EM and immunoblotting. MDA was present on cell membranes, within GEC vesicles and associated with GBMs. Type IV collagen was a recipient of MDA adducts as shown by autoradiography of immunoprecipitates of isolated GBMs from proteinuric PHN rats which were specifically H^3-labelled in their MDA-derivatised proteins. Anti-MDA antibody bound to type IV collagen immunoprecipitated from glomerular extracts. Collagenase treatment of GBM extracts indicated that the NC-1 domain of type IV collagen was a site of adduct formation. Inhibition of LPO *in vivo* by pretreatment of rats with the lipophilic antioxidant probucol markedly abrogated proteinuria compared to controls in both models: (PHN: 24.8 ± 8.0 versus 161 ± 24 mg per 24 hrs; $P = < 0.05$; AN 28 ± 6 versus 186 ± 8 mg per 24 hrs; $P < 0.01$). The glomerular IF signal for, and whole kidney [MDA] ($P < 0.01$) were markedly reduced compared to controls. These findings strongly implicate LPO in the production of the altered glomerular permselectivity that results in proteinuria in PHN and AN.

Expression of the SGP-2 gene in renal regeneration—Continuing evidence for links with apoptosis. *G.C. Gobé*, R. Buttyan#, K.R.L. Wyburn, M.R. Etheridge and P.J. Smith, Dept. of Pathology, Medical School, University of Queensland, Brisbane, 4006 and #Dept. of Urology, Columbia University, New York 10032, USA.* The sulphated glycoprotein-2 (SGP-2) gene has a role in acute and chronic renal injury that is not well understood. Its increased expression is often associated with presence of apoptosis, but some reports demonstrate links with the renal regenerative response found after toxin- or ischaemia-induced acute tubular necrosis. We have used a novel rodent model of renal regeneration to study SGP-2 expression and intrarenal distribution. The model involved initial development of unilateral non-infarctive renal atrophy using experimental renal artery stenosis, followed by contralateral nephrectomy after several weeks. Regeneration of remnant atrophic kidneys was rapid and involved renal cellular hyperplasia rather than hypertrophy. At the time of induction of regeneration, cellular pathology of atrophic kidneys comprised regions of shrunken de-differentiated tubules, an expanded interstitium, and focal hypertrophied but otherwise normal tubules. Apoptosis, which is known to play a major role in earlier development of renal atrophy, was rarely visible, and there was little inflammation. Regenerating renal tissue from male rats ($N = 2$ or 3) was studied at 0, 4, 8, 24 hours, 2, 3, 5, 7, and 14 days after contralateral nephrectomy. Control animals were sham-operated ($N = 2$). Several parameters were studied: level and localisation SGP-2 expression (Northern blots and *in situ* hybridisation); cell proliferation (PCNA immunolocalisation and histological identification of mitosis); and apoptosis (morphological characteristics). During the acute regenerative phase (first 24 hours) expression of SGP-2 was increased, decreasing to untraceable levels by 3 days. Expression was localised most often in de-differentiated atrophic tubules, or the periphery of vessel walls. PCNA counts peaked at 5 days, with epithelial cells of de-differentiated atrophic tubules again most active. Despite the regenerative stimulus, an unexpected but marked transient increase in apoptosis was found in atrophic tubules in the first 2 days. Thus, the results provide evidence that increased expression of SGP-2 is associated with apoptosis rather than regeneration, but a role in restructuring of the atrophic tissue cannot be ruled out. The results also suggest that this model may be useful for studying other early genetic changes found in renal neoplasia.

Regulation of the integrin fibronectin receptor $\alpha_5\beta_1$: Implications for mononuclear cell accumulation at sites of inflammation. *Randall J. Faull* and Mark H. Ginsberg, Department of Renal Medicine, St. George Hospital, Kogarah, NSW, 2217, and Committee on Vascular Biology, The Scripps Research Institute, La Jolla, California.* Disorders dependent on mononuclear cell accumulation at inflammatory sites are frequently encountered in nephrology. They include rejection of allografts, vasculitis, glomerulonephritis, tubulo-interstitial nephritis and pyelonephritis. Cells migrating from the blood to these sites undergo repeated cycles of attachment/detachment to the vascular endothelial cells and the extracellular-matrix. Principal among the cell surface receptors mediating these interactions are members of the integrin superfamily of adhesion molecules, including the "classical" fibronectin receptor $\alpha_5\beta_1$. We have studied the regulation of $\alpha_5\beta_1$ function on mononuclear cells in order to assess its contribution to this process of extravasation and accumulation. A monoclonal antibody (8A2) directed against the β_1 chain directly activated the $\alpha_5\beta_1$ receptors, converting them from a conformation with low affinity for soluble fibronectin (Kd > 1 μM) to one with high affinity (Kd 40 nM). In the presence of soluble fibronectin at a concentration found in normal plasma (700 nM), these high affinity receptors were occupied and unable to mediate adhesion of T lymphoid cells to immobilised fibronectin. In contrast, stimulation of protein kinase C in T lymphoid cells with phorbol myristate acetate increased adhesion to fibronectin by inducing cell spreading. As the receptors remained in a low affinity conformation, the increase in adhesion was not blocked by soluble fibronectin. The findings suggest that only the low affinity receptors are available for adhesion in the presence of physiological concentrations of soluble fibronectin. Both forms of the $\alpha_5\beta_1$ are presumably physiologically relevant, as differentiation of monocytoid cells (THP-1) into adherent, macrophage-like cells induced a subpopulation (30%) of high affinity $\alpha_5\beta_1$ receptors. We hypothesize that: (1) mononuclear cells use the low affinity $\alpha_5\beta_1$ receptors for the reversible interactions required during migration into inflammatory foci; and (2) the high affinity receptors induced by the subsequent differentiation bind soluble fibronectin, enabling the cells to assemble a stabilising fibronectin matrix and hence persist at those sites.

Glomerular expression of ICAM-1 and VCAM-1 in ExHC rats: A model for lipid-induced glomerular injury. *M. Hattori[1,3], D.J. Nikolic-Paterson[1], H.Y. Lan[1], P.A. Hill[2], H. Kawaguchi[3], K. Ito[3], R.C. Atkins[1], Dept. of Nephrology, Monash Medical Centre[1]; Dept. of Anatomy, University of Melbourne[2]; and Dept. of Pediatric Nephrology, Tokyo Women's Medical College[3].* A number of studies have demonstrated an important role for macrophages (Mϕ) in lipid-induced glomerular injury; however, little is known of the mechanisms which mediate Mϕ infiltration in this disease. The present study examined whether adhesion molecules ICAM-1 and VCAM-1 may be involved in glomerular Mϕ infiltration in ExHC rats—a strain which is susceptible to lipid-induced glomerular injury (*Nephron* 63:314, 1993). Twenty-five male 6 week old ExHC rats were placed on a normal diet supplemented with 3% cholesterol, 0.6% sodium cholate and 15% olive oil (HCD). Groups of 5 rats were killed after 3 days, 1, 2, and 6 weeks on HCD diet. A group of 5 matched ExHC rats on a normal diet served as a control. HCD rats showed marked hypercholesterolemia in the absence of any increase in plasma triglyceride levels from day 3 (190 ± 14 vs. 41 ± 3 mg/dl normal; $P < 0.01$), and developed mild proteinuria (21.9 ± 2.7 vs. 5.2 ± 0.5 mg/24 hours normal; $P < 0.01$) and segmental glomerular lesions at week 6. Immunoperoxidase staining identified a significant increase in glomerular ED1+ Mϕ at week 1 which was further increased at week 6 (6.9 ± 0.4 vs. 1.0 ± 0.1 ED1+/gcs normal; $P < 0.01$). There was also a significant increase in glomerular cells expressing the adhesion molecule ligands LFA-1 and VLA-4. Coincident with Mϕ infiltration there was an increase in glomerular anti-ICAM-1 staining in both the number of positive cells and the intensity of staining. Northern blot analysis of cortical RNA found an increase in both ICAM-1 and VCAM-1 mRNA from day 3 onwards. In conclusion, these results suggest that upregulation of ICAM-1 and VCAM-1 expression may play an important role in lipid-induced glomerular Mϕ infiltration.

Plasminogen activator inhibitor type-1 in rabbit crescentic glomerulonephritis: Assessment of function and association with fibrin deposition. *J.H. Erlich*, J. Malliaros, J.A. Apostolopoulos, S.R. Holdsworth & P.G. Tipping, Department of Medicine, Monash University, Clayton, Vic 3168, Australia.* Plasminogen activator type-1 (PAI-1) is a naturally occurring rapidly acting inhibitor of the plasminogen activators—tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). Endothelial cells are a major source of PAI-1. The role of PAI-1 in antiglomerular basement membrane (anti-GBM) glomerulonephritis (GN) was investigated in a rabbit model of anti-GBM GN characterised by: proteinuria, renal impairment, crescent formation, fibrin deposition and macrophage influx. Rabbits were presensitised with a subcutaneous injection of normal horse immunoglobulin followed 5 days later by 25 mg/kg of horse anti-rabbit-GBM antibody intravenously. Renal tissue was collected after 1, 4, 7, and 12 days. Fibrin deposition in isolated rabbit glomeruli was assessed following injection of ^{125}I-fibrinogen and by fibrinogen immunofluorescence in rabbit renal cortical sections. At each time point PAI-1 activity was assessed in conditioned medium of cultured glomeruli using a urokinase functional inhibition assay and PAI-1 mRNA expression was determined using RNA isolated immediately from glomeruli. PAI-1 activity in glomeruli was increased on days 4 (291 ± 18 au) (mean ± SEM) and day 7 (213 ± 27 au) ($P < 0.05$ cf normal 112 ± 38 au). This correlated with an increase in glomerular PAI-1 mRNA which was also increased on days 4 (123.4 ± 7.3) and 7 (133 ± 2.6) ($P < 0.084$ day 4 cf normal; $P < 0.021$ day 7 cf normal). The increase in PAI-1 activity and mRNA showed the same temporal pattern as glomerular fibrin accumulation and macrophage infiltration on days 4 (21.8 ± 3.3 $\mu g/10^3$ glomeruli) and day 7 (20.0 ± 2.5 $\mu g/10^3$ glomeruli) ($P < 0.05$ cf normal 1.5 ± 0.5 $\mu g/10^3$ glomeruli). This close temporal correlation between glomerular PAI-1 function, mRNA expression, fibrin deposition and macrophage influx suggest a role for PAI-1 in glomerular fibrin accumulation. The augmentation of glomerular PAI-1 may be directed by macrophages.

Lipid peroxidation in puromycin aminonucleoside (PAN) induced nephropathy. *J.P. Fawcett, R. Jiang, R.J. Walker*, Medicine Dept., Otago Medical School, Dunedin, NZ.* Reactive oxygen species producing lipid peroxidation have been implicated in the pathogenesis of acute PAN-induced nephropathy. Proteinuria has been used as the marker of glomerular injury. No studies have determined the relationship/time course of lipid peroxidation with the development of proteinuria. Rats were treated with a single IV injection of PAN (7.5 mg/kg). 24 hour urine protein excretion (mg/24 h) was determined prior to sacrifice on days 2, 3, 5, 7, 10, 17, 27, 41 (N = 5–10 per group). The kidneys were removed and flushed with ice cold TRIS buffer. Kidney cortices were homogenised individually. Tissue lipid peroxidation (TBARS ng/mg protein) was measured in whole homogenates and lipid extracts by the thiobarbituric acid assay (HPLC & spectrophotometric determination). N = 5–10 rats, * $P <$ 0.05 vs. control.

Day	0	2	3	4	5	7	10	17	27	41
Tissue peroxidation										
Mean	58.4	94.1*	114*	128*	96.5*	81.1*	77.5	70.8	73.9	78.6
SEM	2.6	2.6	6.6	6.8	4.5	6.8	3.9	5.2	6.4	4.9
Tissue-lipid extract peroxidation										
Mean	29.7	36.4*	48.0*	37.0*	32.6*	27.1	34.7	24.7	29.2	22.3
SEM	6.0	3.6	2.3	5.1	5.1	4.6	2.9	6.5	6.6	2.2
Proteinuria										
Mean	1.2	1.3	1.9	66*	102*	220*	203*	121*	15.3*	10.7*
SEM	0.4	0.2	0.6	13	14	30	20	13	5.3	4.8

Peak lipid peroxidation occurred 48 hours prior to the development of proteinuria. This study supports the role of lipid peroxidation mediating the glomerular injury in PAN nephropathy and defines the relationship between peroxidative injury and the development of proteinuria.

Energy expenditure in children with chronic renal failure. *E.E. Reed*, J.C. McCauley, L.P. Roy, K.J. Gaskin and J.F. Knight, Department of Nephrology and James Fairfax Institute of Paediatric Clinical Nutrition, The Children's Hospital Camperdown, NSW, Australia, 2050.* Energy intake is often poor in children with chronic renal failure. In order to provide optimal nutrition, it is necessary to know the energy requirements of this group. The aim of this study is to determine the resting energy expenditure (REE) in a group of children with glomerular filtration rate (GFR) <30 ml/min/1.73 m^2 and to compare this with the predicted REE according to

Schofield[1]. REE was measured with a flow through ventilated hood open circuit indirect calorimeter in six children after an overnight fast. Energy intake, measured by a 4 day weighed food record, was compared to WHO/FAO recommended energy intake for age. Mean age was 10.8 years, mean height standard deviation score (SDS) was −1.75 and mean weight SDS was −1.06. The mean REE was 98.1% of the predicted REE. Mean energy intake was 88.6% of recommended. Resting energy expenditure is normal in these children with chronic renal failure. However energy intake is below that recommended for children with a normal REE. Malnutrition and growth failure may result if energy intake is not increased to meet energy expenditure. Nutritional support for children with renal failure should be planned to provide the recommended energy intake for age.

1. SCHOFIELD WH, ET AL.: Basal metabolic rate—review and prediction. *Human Nutrition: Clinical Nutrition* 39C:1–96, 1985

Amyloidosis among Central Australian Aborigines. *M.G. Kirubakaran*, N. Rajabalendran and L. Mollison, Department of Medicine, Alice Springs Hospital, Alice Springs, NT—0870.* The incidence of amyloidosis varies greatly between different ethnic groups throughout the world and it is particularly high in the Aboriginal population of Central Australia. Nineteen new cases of amyloidosis were detected during a six year period among a population of 13,200 Aborigines in Central Australia, giving an incidence of 240 cases per million per year. The mean age was 43.5 years (range 26–72) and 11 were females. The main presenting symptom was acute abdomen with pseudo-obstruction in 12 cases, five of whom had exploratory laparotomy prior to diagnosis. Multiple organisms were grown from the peritoneal fluid in all 5 cases. The diagnosis of amyloidosis was established by renal biopsy in 9 cases, rectal biopsy in 7 and at post mortem in 3. The amyloid was AA type in all cases but chronic infection was present only in 4 cases (pulmonary tuberculosis, chronic osteomyelitis, lepromatous leprosy and extensive donovanosis—1 case each). Eleven patients have died, 8 within 6 months of onset of symptoms. Overwhelming infection with multisystem failure was the main cause of death, aggravated by malabsorption and malnutrition. Post mortem findings were similar all cases available for autopsy with widespread amyloid deposits in all the internal organs. End stage renal failure developed in 8 patients, all of whom were commenced on dialysis, and two have been transplanted. The Central Australian Aborigines have a very high incidence of AA type of amyloidosis and the aetiology remains obscure in most of them. The disease appears to have a fulminant course with high mortality and morbidity.

Validation of screening of children for renal abnormalities. *S.J. Harris*, M.P. Dixit, R.J. Hogg, P.H. Henning, N. Wigg^ & K.F. Jureidini, Renal Unit, Women's & Children's Hospital and Child Adolescent Family Health Services^, Adelaide, South Australia 5006.* We have previously screened 8764 kindergarten children for renal abnormalities by measuring BP, growth percentiles and testing frozen first morning urine for blood, protein, glucose, osmolality, nitrites and β_2-microglobulin. 978 (11.3%) of these showed abnormalities and were reviewed in the renal clinic with full history and examination, repeat urine assays and renal ultrasound, where indicated. Of these, 86 (8.8%) were ultimately shown to have significant abnormalities. Screening has been performed on a further 690 kindergarten children. Of these 77 (11.3%) initially screened abnormal and 11 (14.3%) were ultimately proven significantly abnormal. 347 children, normal on initial screen, were recalled. On review in the renal clinic, 2 (0.6%) were proven to have significant abnormalities (one had dominant polycystic kidneys with a positive family history, normal BP and urinalysis and the other a normotensive co-arctation of the aorta with obviously diminished femoral pulses on examination). The study shows a significant difference in ultimate diagnosis of renal abnormalities between those showing changes on the primary screen compared with those who do not (8.8% cf 0.6%, $P < 0.001$). This, coupled with the similar abnormality rate in this recent screening compared with the previous larger study, suggests the validity of our screening methods.

Haematuria and urinary red blood cell morphology. *P. Sizeland*, R.R. Bailey, B. Harris, Departments of Nephrology and Microbiology, Christchurch Hospital.* Urinary red blood cell (rbc) morphology is widely used in the evaluation of haematuria. Some concerns persist with the technique, however, including the lack of a precise classification of rbc morphology and the proportion of cells with abnormal morphology that constitutes glomerular haematuria. Using a recently described morphological classification (Tomita et al., *Clin Nephrol* 37:84–9, 1992), we evaluated the technique in the differential diagnosis of urinary tract haematuria. Results are shown in Table 1 (N = 108, 40 glomerular, 68 non-glomerular, Table 1):

Table 1

	Correct	Incorrect
Glomerular	32	5
Non-glomerular	63	8
Sensitivity 80%	Specificity 93%	

Using readily available laboratory results (proteinuria, casts, plasma creatinine), in addition to urinary rbc morphology, improved precision as shown in Table 2:

Table 2

	Correct	Incorrect
Glomerular	36	5
Non-glomerular	63	4
Sensitivity 90%	Specificity 93%	

We conclude that:—(1) urinary rbc morphology is a useful but not definitive tool in the assessment of a patient with haematuria, (2) urinary rbc morphology should be used in conjunction with other available laboratory results.

Childhood factors in aboriginal renal disease. **D.A. McCredie, B. Craig, W. Hoy, J.D. Mathews and R. McFarlane, Menzies School of Health Research and Royal Darwin Hospital, PO Box 41096, Casuarina, Northern Territory 0811, Australia.* Children from an aboriginal community in the far north of Australia are being followed-up as part of a longitudinal study to investigate possible aetiological factors in the development of chronic renal disease, which affects up to half of the adult population of this community. Data on 219 children, aged 6 to 16 years, have been collected, representing three quarters of the school-age population, with particular reference to nutrition, history of infections, presence of skin sores, blood pressure, urine examination, bacterial isolation from skin and throat and streptococcal antibody titres. Significant haematuria on dipstick testing was found in 31 children (14%) and proteinuria in nine (4%), with abnormal urine albumin creatinine ratios (>5 mg/mmol) in seven. Only two children had both blood and protein in urine. Malnutrition by European standards, and a high infection rate (particularly of chest and skin) were prevalent, especially in early life, but showed no correlation with haematuria or proteinuria. Likewise there was no correlation between indices of renal disease and isolation of Group A Streptococcus pyogenes (25% of children), high streptococcal antibody titres (almost universal), or a past history of post-streptococcal glomerulonephritis (26 children). The only significant correlation was that of proteinuria with age. Only three of 160 children less than 13 years had proteinuria, compared with six of 59 aged 13 to 16 years [$P < 0.01$]. This proportion increases to 27% in the third decade of life, and reaches almost 50% by the age of 40 years. These preliminary results suggest that proteinuria, implying significant renal disease, is becoming appreciable by the early teenage years though a relationship to the presence of haematuria in earlier years has not been shown. Follow-up of these children is continuing in the hope of identifying predictive factors in the development and progression of renal disease.

Aerotolerant coryneforms as urinary tract pathogens. *R.R. Bailey*, B. Harris, Departments of Nephrology and Microbiology, Christchurch Hospital, Christchurch.* Most urinary tract infections in clinical practice are due to Enterobacteriaceae. It is well documented that mycobacterial, fungal, viral and other atypical organisms may invade the urinary tract. Recently we have seen a number of patients with unequivocal colonisation of the urinary tract with an aerotolerant coryneform. All had heavy pyuria. The clinical details are included in the table.

No.	Age	Sex	Underlying problem	Presentation
1	42	F	Reflux nephropathy	Acute pyelonephritis
2	63	M	Diabetes, urethral stricture after TURP	Asymptomatic
3	83	F	Large residual urine	Asymptomatic
4	85	M	Myelofibrosis, renal failure, TURP	Asymptomatic
5	78	M	Recurrent UTIs	Asymptomatic
6	72	F	Demented, incontinent	Asymptomatic

The finding of aerotolerant coryneforms growing in voided urine immediately suggests contamination during the collection process. However, in 4 of these 6 patients there was unequivocal colonisation of the urinary tract proven with urine obtained by suprapubic aspiration or catheterisation. Although 5 of these patients were elderly, none had any evidence of associated bowel or gynaecological problems. If patients have a urinary tract abnormality and pyuria and nothing is cultured with conventional techniques, then consideration should be given to culturing for this group of pathogens.

Prednisolone (PNL) decreases haematuria in IgA nephropathy. *K. Nicholls*, P. Kincaid-Smith, G. Becker, Dept. of Nephrology, Royal Melbourne Hospital, Parkville 3050.* Thirty-three patients with biopsy proven IgA nephropathy and urinary erythrocyte count (URBC) consistently $>10^5$ on 3 occasions over 3 months entered a prospective controlled trial of PNL therapy. Patients were randomly allocated to no additional therapy or PNL (25 mg daily, for 4 weeks, decreasing to 12.5 mg daily by 6 weeks, then to 10 mg daily from 6–12 months). All patients were reviewed at 1, 3, 6, 9, 12 & 15 months, and clinical parameters, urinary erythrocyte counts, proteinuria and creatinine clearance were recorded. Tc-DTPA renal scans with GFR were obtained at the beginning and end of the study. Four patients (3PNL) were withdrawn during the study, for steroid induced diabetes 1, non-compliance 2, Cr ≥0.50 mmol/l 2. Both patient groups were well matched for age, renal function and URBC at entry. *Results:*

	Median URBC × 10^5/mL	
TIME	PNL	No PNL
0	4.0	3.5
1 month	1.2*	4.1
3 months	1.3	2.0
6 months	1.1	1.6
9 months	1.1	1.9
12 months	.64*	2.2
15 months	1.9	1.4

* $P = 0.03$ Wilcoxin

No effect of PNL on renal function or proteinuria was demonstrated. We conclude that PNL significantly decreases URBC, but that this effect is not sustained as the dose is decreased to acceptable maintenance levels.

Oxidisability of low density lipoprotein in patients on renal replacement therapy. *K.C. Wong[1], M. Luo[2], R. O'Brien[2], N. Balazs[3], C.J. Wood[1], P.G. Kerr[1], R.C. Atkins[1], Depts. of Nephrology[1]/Medicine[2]/Biochemistry[3], Monash Medical Centre, Clayton Vic 3168.* In vitro evidence suggests that oxidative modification of Low Density Lipoprotein (LDL) plays an important role in atherogenesis. The potential influence of treatment modality for renal failure on the oxidisability of LDL has not been studied previously. We measured the susceptibility of LDL to oxidation in 27 healthy controls (C), 17 CAPD patients (PD), 20 haemodialysis patients (HD) and 19 kidney transplant recipients (KidTx) using in vitro oxidation with copper. This is expressed as lag time (min) between addition of copper and commencement of oxidation. The change in Absorbance represents conjugated diene formation and correlates with the presence of products of oxidation. Thus, a long Lag phase and low ▲ Absorbance are associated with a lower risk of atherogenesis. HD and PD patients had higher Triglyceride levels than C ($P = 0.03$, $P = 0.01$ respectively). KidTx had lower Triglyceride levels than PD ($P = 0.01$) and a trend for lower Triglyceride levels than HD. KidTx had higher Cholesterol levels ($P = 0.001$) when compared with HD. There was no difference in the Lag time between HD, PD, and C. HD had increased products of oxidation as measured by ▲ Absorbance ($P = 0.0002$ cf C and $P < 0.0001$ cf PD). It is possible that the Haemodialysis procedure itself may contribute to a lower antioxidant state and thus result in increased lipid peroxidation in this group of patients. With the restoration of renal function after transplantation, the elevated ▲ Absorbance returns to normal. This may contribute to a reduction in atherogenicity and vascular disease.

Cholesteryl ester transfer (CET) activity in patients with chronic renal failure (CRF). *J. Corboy, W.H.F. Sutherland, R.J. Walker*, M.C. Robertson, C.M. Cox, Department of Medicine, Otago Medical School, Dunedin, NZ.* Patients with renal failure have an increased risk of atherosclerosis in part due to lipid and lipoprotein abnormalities. Alterations in reverse cholesterol transport may contribute to the increased risk. CET protein catalyses the redistribution of newly synthesised cholesteryl esters (CE) from HDL to other apo-B lipoproteins. The effects of renal therapy on CET activity have not been fully studied. Plasma CET was determined by measuring the accumulation in apo-B lipoproteins of CE generated from plasma free 3H-cholesterol. 53 patients (ages 15–77) were studied. 13 were dialysis independent, 12—haemodialysis, 11—CAPD, 17—renal transplants.

Plasma CET rate (nmol/ml/hr)

	DI	HD	CAPD	RT	Control
Normolipidaemic	20.7 ± 2.7	19.5 ± 4.2	22.0 ± 3.6	22.9 ± 6.2	18.0 ± 5.6
Hyperlipidaemic	27.6 ± 9.7	29.2 ± 13.0	36.3 ± 12.5	32.4 ± 11.7	27. ± 7.6
Total	25.5 ± 10.5	24.4 ± 10.5	32.4* ± 12.5	27.9 ± 10.4	22.1 ± 7.9
Apo B (g/l)	0.97 ± 0.31	0.95 ± 0.32	1.33** ± 0.34	0.99 ± 0.3	0.87 ± 0.2

* $P < 0.05$ ANOVA with control; ** $P < 0.01$ ANOVA compared to all groups

Plasma CET rate was higher in hyperlipidemic subjects with the highest rates in the CAPD group. In the normolipidemic subjects there was no difference compared to controls. Plasma apo-B levels correlated with CET rates in all renal patients ($r = 0.754$ $P < 0.001$). This study shows that plasma CET rate is closely associated with apo-B and lipid levels irrespective of treatment modality. Raised CET activity may not be inevitable in CRF as activity was normal in absence of hyperlipidemia. What influence lipid lowering therapy may have on plasma CET activity in hyperlipidemic patients with CRF warrents further investigation.

Lipoprotein (a) and other potentially atherogenic lipids in maintenance dialysis and renal transplantation. *L.S. Ibels*, C.A. Pollock, C.S. Ong, R.J. Caterson, D.A. Waugh, J.F. Mahony, Department of Renal Medicine Royal North Shore Hospital, St. Leonards, 2065, Australia.* Serum lipids and lipoprotein (a) concentrations were measured in 91 renal transplant and 60 dialysis patients, and correlations sought with manifest vascular disease. Serum lipoprotein (a) concentrations were greater than 300 mg/l in 24% of the renal transplant recipients and 40% of the dialysis patients, and other potentially atherogenic lipid abnormalities were very prevalent. In the renal transplant recipients, lipoprotein (a) correlated with age ($P < 0.001$) and inversely with azathioprine dosage ($P < 0.01$). Low HDL cholesterol concentrations ($P < 0.05$) and high total cholesterol to HDL cholesterol ratios ($P < 0.01$) were more strongly associated with the presence of vascular disease than was elevated lipoprotein (a). In the dialysis patients, a low serum albumin ($P < 0.05$) and a low serum creatinine ($P < 0.001$), indicative of a poor nutritional state, were associated with the presence of vascular disease. A high total serum cholesterol to HDL cholesterol ratio ($P < 0.05$) was indicative of ischaemic heart disease, and a high total serum cholesterol ($P < 0.01$) and LDL cholesterol ($P < 0.01$) of cerebrovascular disease. Although patients on CAPD ($N = 21$) tended to have higher serum lipoprotein (a) compared with patients on haemodialysis ($N = 39$), i.e. 403 ± 76 vs. 260 ± 37 mg/l, the differences were not significant ($P = 0.11$). In CAPD patients, serum lipoprotein (a) correlated with total serum cholesterol ($P < 0.05$) and the LDL subfraction ($P < 0.05$). Elevated serum lipoprotein (a) levels were associated with cerebrovascular disease in the CAPD patients ($P < 0.01$).

In both the transplant and dialysis populations no associations of serum lipoprotein (a) were seen with sex, duration of dialysis prior to transplantation, presence of diabetes mellitus or smoking. The present study demonstrates that an elevation in serum lipoprotein (a) concentration is very prevalent in patients on renal replacement therapy, but is not as strongly associated with the presence of vascular disease as are the total serum cholesterol, HDL and LDL cholesterol levels and the ratio of total cholesterol to HDL cholesterol.

Successful self-care home dialysis in the elderly: A single centre's experience. *M. McDonald, P.D. McPhee, R.J. Walker, Department of Nephrology, Dunedin Hospital, Dunedin, New Zealand.* The increasing number of older people in the population has been associated with an increased incidence of renal failure and higher acceptance rates for renal replacement therapy in New Zealand and Australia. We have analysed prospectively our unit's experience with renal replacement therapy in the 60 years and older age group since the introduction of CAPD in 1989. There were 25 patients (21 Caucasians, 3 Maori, 1 Cambodian—16 males, 9 females) with an average age of 64.4 years (range 58.25–76.5 years at the commencement of dialysis). They made up 34% of the dialysis population and all were on self care home dialysis. At the completion of the study 17 of the 25 patients were still alive; 2 with functioning transplants, 3 on hemodialysis and 12 on CAPD. The duration on dialysis ranged from 8 to 71 months with a median of 35 months. Patient survival rates were comparable with the national average at 12 months (90% vs. 89%) and 2 years (84% vs. 80%). During the study there were 8 deaths, 6 due to cardiac causes, one due to multiple myeloma and one withdrawl from therapy. Dialysis therapy was well tolerated and technique survival rates were comparable for both hemodialysis and CAPD. At 12 months CAPD technique survival rate was 80%, 70% at 2 years and 50% at 3 years. For hemodialysis the technique survival rate was 83% at 12 months, 73% at 2 years and 36% at 3 years. The CAPD peritonitis rate was 1 in 28.5 patient months. Self care home dialysis is a viable therapeutic option with a high degree of compliance and good quality of life in the older population.

A survey of renal disease in a Northern Territory (NT) Australian Aboriginal (AA) community. *Wendy Hoy*, John Mathews, Robert McFarlane[1], David Pugsley[2], Menzies School of Health Research and Royal Darwin Hospital[1], Casuarina, NT 0811 and the Queen Elizabeth Hospital, Woodville, SA 5011[2].* We screened 262 adults (43% of the 1991 adult census) in a NT AA community, to clarify rates and associations of the glomerulopathy underlying their high rates of renal failure. Albumenuria was quantitated by the albumen/creatinine ratio (ACR, gm/mole), with 3.4–33.9 (30–299 mg/gm) indicating microalbumenuria, and 34+ (≥300 mg/gm) indicating overt albumenuria. Glucose tolerance (GT) was defined by WHO criteria. BMI exceeded 25 kg/m^2 in 29.7% of subjects; 19.6% had type 2 diabetes, and 10.7% had impaired GT (IGT); 18% of females and 38% of males had SBP ≥140 and/or DBP ≥90 mm Hg. Fourteen subjects had leukocyturia, and 4 had urinary infections. ACRs, similar in males and females, were as follows:

	<3.4	3.4–33.9	34+
Normal glucose tolerance (182)	56.0%	23.6%	20.8%
IGT and chemical diabetes (47)	36.1%	29.8%	34.0%
Clinical diabetes (33)	12.1%	18.2%	69.7%

Glucose tolerance was normal in 66% of subjects with microalbumenuria and 49% of those with overt albumenuria. Blood pressures were normal in 81% of subjects with microalbumenuria and 51% with overt albumenuria. ACRs rose with age, as shown for subjects with normal GT:

	<3.4	3.4–33.9	34+
17–34 years (117)	74.3%	14.5%	11.1%
35–54 years (48)	39.6%	31.3%	29.2%
55+ years (17)	11.8%	47.0%	41.2%

Serum creatinine was elevated in 6.9% of subjects (<106 mmole/L in females, >120 in males), all with overt albumenuria. Idiopathic hematuria ≥1+ by dipstick was present in 6.5%, 10.2% and 20.6% of subjects in the three ACR categories, or 12.9% overall. Subjects with multiple skin sores had a 60% increase in hematuria (NS, $P = 0.26$), with no further increase in the presence of group A streptococci, even those of nephritogenic M types. Skin sores did not correlate with ACR. These data indicate high rates to renal disease which is not consistently marked by hematuria and is only variably complicated by hypertension; its expression increases with age, and might be exacerbated by IGT and diabetes. Its aetiology, pathogenesis and natural history are being pursued, and a treatment trial is planned.

Plasma interleukin-6 and stress hormone responses to acute pyelonephritis. *R.A. Donald, R.R. Bailey*, D.N.J. Hart, J.H. Livesey, M.J. Evans, L. Mattioli, J. Macdonald, A.H. Smith, Departments of Endocrinology, Nephrology and Immunology, Christchurch Hospital, Christchurch.* This study was undertaken to investigate the relationships between the "stress hormones" corticotrophin (ACTH), vasopressin (AVP), corticotrophin releasing hormone (CRH) and cortisol, and the cytokines, interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor (TNF) in patients hospitalised with acute pyelonephritis (fever > 38°C, loin pain or tenderness, infected urine). Ten patients (7 female; age range 16–56 yr) with normal renal function were studied. Peptide hormones were measured by RIA; cortisol and cytokines by ELISA. Reference ranges were from samples donated by ≥40 volunteers from the electoral roll. Mean serum IL-6 and plasma AVP and CRH concentrations on admission were significantly raised above mean 0800 hr values for normal volunteers ($P < 0.001$), but mean plasma ACTH and cortisol were not. Mean IL-6 and AVP were more than 2 SDs above the mean reference range for 72 hours, although IL-6 tended to fall after 24 hr. No change in serum IL-1 and TNF were observed in 3 patients. The correlation between the IL-6 and cortisol concentrations at all sampling times and in all subjects was highly significant ($P < 0.001$). Significant correlation between serum IL-6 and AVP ($P < 0.005$) and IL-6 and ACTH ($P < 0.05$) were observed. No correlation between IL-6 and CRH could be demonstrated. In summary, mean circulating levels of IL-6, AVP and CRH were significantly raised in hospitalised patients with severe acute pyelonephritis. Plasma concentrations of IL-6 correlated well with the 'stress hormones' cortisol, ACTH and AVP, but not CRH.

The influence of native and modified low density lipoproteins on normal and diabetic rat glomerular core production of eicosanoids: The role of phospholipase A2. *N. Harley*, M. Dunlop, G. Becker, R. Larkins, Departments of Medicine and Nephrology, University of Melbourne, The Royal Melbourne Hospital, VIC.* Hyperlipidaemia is associated with a variety of renal diseases, particularly diabetic nephropathy, and experimental evidence suggests a role for lipoproteins (LP) in its progression. The influence of low density lipoproteins (LDL), unmodified and modified by oxidation (OXLDL) or by glycation (GLCLDL), on prostaglandin E2 (PGE2) and thromboxane A2 (TxA2) production by mesangial cell enriched glomerular cores (GC) and the contribution of cell associated phospholipase A2 (PLA2) was investigated. Native and modified LDL was prepared from human plasma. GCs were prepared by differential sieving and collagenase digestion from control and Streptozocin (STZ)-injected Sprague-Dawley rats with diabetes of 3 weeks duration. GCs were incubated with the indicated LP for one hour. PGE2 and TxA2 (as TxB2) were assayed by specific radioimmunoassay and expressed as pg/100 μg protein/hr. († $P < 0.05$ †† $P < 0.01$ vs. non-diabetic control, * $P < 0.05$, ** $P < 0.01$ vs. LP free).

	control	μg/ml LDL		OXLDL	GLC LDL
PGE2	1.38 ± 0.11	50	2.44 ± 0.34*	11.65 ± 1.22**	4.91 ± 0.34**
		100	2.47 ± 0.32**	13.73 ± 1.17**	5.20 ± 0.65**
TxB2	0.31 ± 0.02	50	0.41 ± 0.04	5.15 ± 0.27**	0.63 ± 0.09*
		100	0.84 ± 0.16*	11.33 ± 0.78**	0.65 ± 0.09*
STZ rats					
PGE2	4.58 ± 0.11††	50	3.24 ± 0.49	11.73 ± 0.60**	4.89 ± 0.38
		100	4.12 ± 0.33	35.17 ± 8.38*	8.23 ± 0.84*
TxB2	0.73 ± 0.07†	50	0.93 ± 0.18	4.78 ± 0.06**	1.04 ± 0.04*
		100	1.12 ± 0.17	10.52 ± 0.72**	1.58 ± 0.16*

After incubation of the GCs for 30 minutes PLA2 activity was measured in vitro by release of arachidonic acid from arachidonyl containing 14C phosphatidylcholine, and activity expressed as nmol substrate hydrolysed/min/μg protein.

	control	μg/ml	LDL	OXLDL	GLC LDL
PLA2	4.52 ± 0.72	100	5.53 ± 0.69	8.94 ± 0.69**	5.12 ± 0.54

It is of particular relevance to diabetic nephropathy that LDL (specifically OXLDL)-induced changes in PLA2 may be responsible in part for alteration in prostaglandin and thromboxane production which in turn may contribute to the altered glomerular haemodynamics implicated in the progression of diabetic renal disease. [Data on PGE2 previously presented ANZSN Hobart 1993]

Effects of growth hormone treatment on insulin-like growth factors and their binding proteins in serum. *S. Harrer*, M.J. van Renen, K.F. Jureidini, and A.A. Martin, Renal Unit, Women's and Children's Hospital, and Child Health Research Institute, 72 King William Road, North Adelaide, S.A. 5006.* It has been clearly demonstrated that growth retardation associated with chronic renal failure (CRF) is significantly alleviated by administration of recombinant human growth hormone (rhGH). The mechanisms of the failure in CRF are not yet fully understood, but increased serum concentrations of insulin-like growth factor binding proteins (IGFBPs) may be involved. The current study involves the analysis of the serum of 9 boys with CRF (aged 4.6–13 years) treated with rhGH (30 IU/m^2/week). We have employed fast protein liquid chromatography (FPLC) under neutral conditions in order to compare the IGF binding protein profiles at stages throughout the treatment period within the same person, and in comparison with normal age- and sex-matched children. Prior to assay of IGF-I and -II by radioimmuno- and radioreceptor assay respectively, the extraction of IGFs was achieved with the use of high performance liquid chromatography (HPLC) under acidic conditions. Serum was also assayed for IGFBP-1, IGFBP-2, and the acid-labile subunit (ALS) by radioimmunoassay. FPLC profiles demonstrated increased IGF-I binding in the 50-30 kDa binding protein region in the serum of CRF children. IGF-I and -II levels in serum rose by 160% and 40% respectively after 12 months of rhGH treatment. At the same time, IGFBP-3 concentrations rose by 97%, while IGFBP-1 fell by 35%. Levels of IGFBP-3 and ALS were strongly correlated throughout the treatment period ($P < 0.01$). This study showed abnormal IGFBP profiles in the serum of children with CRF. rhGH treatment resulted in greater proportional increases in IGF-I than of IGFBP-3, while IGFBP-1 levels fell, suggesting that the efficacy of rhGH in overcoming the growth retardation is, at least in part, due to a relative reduction in IGF binding capacity in serum.

Acute effect of ethanol on rat renal electrolyte transport. *S.L. Carney*, A.H.B. Gillies, C.D. Ray, Discipline of Medicine, University of Newcastle, NSW, 2308.* The direct effect of ethanol on renal water and electrolyte transport is poorly understood despite its widespread use as a recreational drug. In particular data on the acute diuretic effect is contradictory and the less well known antidiuretic effect has not been evaluated. Data on electrolyte transport is sparse and also contradictory. Therefore the acute effect of increasing plasma concentrations of ethanol was evaluated in water diuretic anaesthetized rats which inhibits endogenous AVP release. Ethanol at a plasma concentration of 1.69 ± 0.28 mmol/L produced an immediate increase in urine flow (174 ± 11 to 189 ± 13 and 206 ± 12 μL/min; $P < 0.01$) as well as an increase in fractional sodium excretion (0.17 ± 0.04 to 0.28 ± 0.05 and 0.27 ± 0.05%; $P < 0.01$). There was also a brief phosphaturia. These increases in electrolyte excretion had returned no control values by 20 minutes despite a further increase in the plasma ethanol concentration. The urinary excretion of potassium, calcium and magnesium was not altered nor was glomerular filtration rate or renal plasma flow. Ethanol at a mean concentration of 3.21 mmol/L did not alter the action of a maximal concentration of AVP (75 ng/kg) on water or electrolyte transport. However, the antidiuretic effect of a submaximal concentration of AVP (7.5 ng/kg) was augmented by ethanol. These studies suggest that the ethanol induced diuresis commonly described to inhibition of AVP secretion may also be due to other intrarenal effects of ethanol, possibly acting within the proximal tubule. These results also confirm recent in vitro findings that while ethanol does not inhibit the action of a maximal concentration of AVP, it does modulate the effects of lower AVP concentrations and possibly also distal water transport in the absence of AVP.

A stereological analysis of the developing rat metanephros. *J.F. Bertram*, K. Spencer and G.B. Ryan, Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Vic, 3052.* The development of the permanent kidney, or metanephros, is a complex process. An objective, morphometric description of metanephric architecture should prove useful in identifying the role of specific growth factors in metanephric development. In the present study, stereological methods were used to estimate the absolute volumes of seven compartments in the developing rat metanephros at embryonic days 15 (E15), E17, E19 and E21. Metanephroi from Sprague-Dawley rats were embedded whole in glycolmethacrylate, exhaustively sectioned at 2 μm, and stained with PAS. The left metanephroi of three embryos from each of three mothers were analysed at each of the four ages (a total of 36 kidneys). Volume densities obtained using point counting were multiplied by total metanephric volume (obtained using the Cavalieri principle) to estimate absolute volumes. Estimates were (mean ± SD):

	E15	E17	E19	E21
	Absolute volume (mm^3)			
Mesenchyme	0.0782 ± 0.0329	0.776 ± 0.184	3.965 ± 0.668	6.266 ± 1.048
Ureteric epithelium	0.0071 ± 0.0032	0.110 ± 0.026	0.749 ± 0.118	1.233 ± 0.103
Vesicles and comma-shaped bodies	0.0105 ± 0.0057	0.116 ± 0.040	0.638 ± 0.139	1.036 ± 0.238
S-shaped bodies	0	0.059 ± 0.036	0.345 ± 0.060	0.426 ± 0.070
Glomeruli	0	0.015 ± 0.011	0.388 ± 0.077	0.671 ± 0.073
Tubules	0	0.030 ± 0.020	1.635 ± 0.276	3.627 ± 0.585
Other	0	0	0.105 ± 0.065	0.128 ± 0.056

These data provide a baseline for future studies on the role of specific molecules in renal development.

^{133}Cs NMR evidence for tissue potassium compartmentation. *R.M. Wellard*, W.R. Adam, B.P. Shehan,# D.J. Craik#, Heidelberg Repatriation Hospital, Heidelberg West, Victoria 3089, Australia; #Victorian College of Pharmacy, Monash University, Parkville Victoria 3052, Australia.* This study uses the NMR sensitivity of caesium as a congener of potassium to provide further evidence for intracellular compartmentation of tissue potassium. Following a caesium supplemented diet tissue caesium levels of 62.7 ± 7.4, 74.2 ± 2.9, 19.9 ± 5.0 6.4 ± 2.4 and 0.66 ± 0.26 μmol/g were achieved for liver, muscle, brain, erythrocytes and plasma respectively. While potassium NMR spectra of both liver and muscle show single component spectra, caesium spectra of liver at 65.60 MHz show the presence of two components the separation being up to 0.8 ppm, suggesting the presence of two pools of potassium in liver which are in slow exchange on the NMR time scale. In muscle there was a single peak. This could be due to the absence of distinct pools of potassium in slow exchange or a low proportion of all but one potassium environment. Mitochondria are a possible site for potassium to experience a different environment. NMR spectra of isolated liver mitochondria are also non Lorentzian with two peaks separated by 2.3 ppm. The two peaks seen for mitochondria are probably from the mitochondrial matrix space and the medium, as determined by shift reagent Dy(TTHA)$^{3+}$. The distribution of caesium in the two mitochondrial compartments also changes with time. The peak attributed to matrix space changes from an initial 50% of total mitochondrial caesium to 15% after 6 h. These results add to the evidence for compartmentation of caesium, and thereby potassium, in the cell and show that NMR can be used to show dynamic changes in tissue electrolyte content.

Systemic infusion of vasoactive intestinal peptide (VIP) impairs response to a volume load. *M.A. Lonergan*, S. Aglibut and M.J. Field, Department of Medicine, University of Sydney, Concord Hospital, NSW 2139, Australia.* An impaired renal excretory response to a volume load occurs in cirrhosis. As elevated systemic plasma levels of VIP, due to decreased hepatic metabolism, have been proposed to contribute to the generalised systemic vasodilation that occurs in cirrhosis and result in sodium and

water retention, we conducted clearance studies in normal Sprague-Dawley rats to examine the effect of elevated plasma levels of VIP on the renal response to a volume load. Animals were studied under Inactin anaesthesia. Ringer's solution was infused at 1% of body weight/h following a priming injection of 1%. After an equilibrium period, two baseline clearance periods (20 min each) were performed, following which the Ringer's solution was increased to 4% body weight/h for four clearance periods. During the volume load VIP was infused at 0.1 nmol/100 g/hour. The Ringer's was then reduced back to 1% body weight/h for a further two clearance periods. Time Control animals (TC) received the Ringer's solution alone. Tritiated inulin and ^{14}C p-aminohippuric acid were included in the infused fluids to permit calculation of GFR, fractional electrolyte excretion rates, renal plasma flow (RPF) and filtration fraction (FF). Student's t-test was used to compare results between groups and paired t-test the delta response. Blood pressure fell significantly during the VIP infusion (15.6 ± 3.9 mm Hg) despite the concurrent infusion of a volume load. BP did not change significantly in the TC group (2.3 ± 4.7 mm Hg). Both GFR and RPF fell acutely with commencement of the VIP infusion but then returned to control values. FF was unchanged. Fractional (FENa) and absolute excretion (UNaV) of sodium increased significantly compared to baseline for both groups; however the response of the VIP group remained less than the TC group (ΔUNaV VIP 5.8 ± 1.4 and TC 9.0 ± 0.8 μmol/min per 100 g). Flow also increased over baseline but the VIP group remained significantly less than TC. VIP therefore impairs the response to a saline load in normal animals. This is mediated in part by a fall in systemic blood pressure. These haemodynamic and renal changes are similar to those that occur in cirrhosis. These data thus support the view that VIP may be involved in the altered sodium and water homeostasis that occurs in cirrhosis.

Low protein diet on the long-term effects of warm ischaemic renal injury in the rat. *P. Jablonski[1]*, D. Lau[1], V.C. Marshall[1], A. Stein-Oakley[2], N.M. Thomson[2], Dept. of Surgery[1], Monash Medical Centre, and Dept. of Medicine[2], Alfred Hospital, Monash University.* Low protein diets have been used in patients with chronic renal disease. This study investigates the effect of dietary protein on the progression of renal failure after warm ischaemic injury (WI) in a rat model. WI was produced in male adult inbred Dark Agouti rats (220–300G) by clamping left renal artery, vein and ureter for 90 or 120 minutes and right nephrectomy (Nx) 3 days later. Rats, fed either normal protein (NP = 20% protein, standard pellets, Barastoc) or low protein (LP = 8% protein, an isocaloric pelleted synthetic diet), were followed for 30, 90 or 180 days. All rats survived WI and Nx with initial weight loss. Both diet groups regained weight at a similar rate. Plasma creatinine was significantly ($P < 0.05$) higher in LP rats from Day 8–90. Creatinine clearance was impaired in both LP and NP rats; the impairment was greater in LP rats. Compensatory renal hypertrophy (NP = 75 + 7, LP = 48 + 12% at 180 days) was also significantly impaired in LP compared to NP. Creatinine clearance per gram kidney was similar to control values in all NP and LP rats and there was no difference in interstitial fibrosis between LP and NP kidneys. After 180 days, NP rats showed increased proteinuria compared to LP rats (NP = 49 + 3, LP = 22 + 4 mg/day) but this was within normal limits (25 to 55 mg/day). Cortex and medulla had a significant leucocyte infiltration which diminished with time. After 180 days, the infiltrate was significantly lower in LP kidneys compared to NP kidneys (medulla: NP = 1500 + 30, LP = 1060 + 50 and cortex: NP = 760 + 60, LP = 520 + 50 cells/mm^2). Conclusion: The possible benefits of LP in reducing proteinuria and leucocyte infiltration were outweighed by impairment of compensatory functional renal hypertrophy.

Mechanisms of iron (FE) tubulotoxicity in proteinuric chronic renal failure (CRF). *D.C.H. Harris, J. Chen, L. Chen, B. Nankivell, and Y-C. Tay, Dept. Renal Medicine, Westmead Hospital, NSW 2145.* Lysosomal FE correlates with proteinuria and best predicts tubular damage in the rat remnant kidney (RK) model of CRF. To investigate mechanisms of FE-tubulotoxicity the effect of FE-loading on lipid peroxidation (malondialdehyde, MDA), lysosomal (N-acetyl glucosaminidase, NAG; acid phosphatase, AP) and mitochondrial enzyme activity and stability (enzyme release), FE-reactivity and structural and functional damage was assessed in proximal tubular cell culture (PTCC) and/or subcellular fractions of normal (N) and 4–8 wk RK. Cytotoxicity in PTCC was assessed by trypan blue exclusion, LDH leakage and morphology. In PTCC cytotoxicity of nitrilotriacetate (NTA)-FE was dose (≥50 μM) and time (≥6 hr)-dependent. The EC50 was 400 μM NTA-FE at 24 hrs. Toxicity of transferrin-FE will be examined. Acute and chronic (4 wk) *in vivo* NTA-FE loading increased MDA in cortical cytosolic and lysosomal/mitochondrial subfractions of RK and N (e.g. RK, cytosolic: 1.3 ± 0.2 vs. 2.4 ± 0.03 nmol/mg protein, μ ± SE; $P < 0.01$). At the stage when RK tubular cell damage was evident by electron microscopy, NAG and AP activities were higher in RK than N ($P < 0.001$), and NAG activity was increased further by NTA-FE. Lysosomal fragility of RK and N were similar, and were decreased by NTA-FE. Mitochondrial enzyme activity was lower in RK than N, and NTA-FE had a variable and no effect respectively on mitochondrial enzyme activity and fragility. Thus, impaired function and increased fragility of lysosomes and mitochondria are unlikely mediators of early FE-induced renal damage. FE-tubulotoxicity in proteinuric CRF be mediated by lipid peroxidation.

Changes in IGFBPs in children with chronic renal failure, rhGH treatment and transplantation. *M.J. van Renen, S. Harrer, A.A. Martin, and K.F. Jureidini, Renal Unit, Women's and Children's Hospital, and Child Health Research Institute, 72 King William Road, North Adelaide, South Australia, 5006.* Three prepubertal children with severe chronic renal failure (CRF) and short stature were treated with recombinant human growth hormone (rhGH), at 30 IU/m^2/week for 2 years, each receiving a renal transplant early in the second year. Serum collected before and during therapy was analysed for insulin-like binding proteins (IGFBPs) by Western ligand (WLB) and immunoblotting techniques, and radioimmunoassay (RIA) for IGFBP-3, acid-labile subunit (ALS) and IGFBP-1. Age and sex-matched normal serum was used as control. Ligand binding was noted on WLB at 44–48, 34, 28, and 24 kDa and was identified as an IGFBP-3 doublet, IGFBP-2, IGFBP-1 and IGFBP-4 respectively by immunoblotting. A fainter protein band at 30 kDa was seen on WLB and identified as IGFBP-3 by immunoblotting. This band was also present on immunoblot in the normal controls but was not clearly seen on the WLB. IGFBP-1, not apparently present on immunoblotting in control serum, showed as a dense band in the CRF patients. This band disappeared after transplantation. IGFBP-2 was seen, on WLB, as a more intense band in CRF children prior to transplantation than in the normal controls. This band also virtually disappeared following transplantation. In the first year on rhGH treatment, mean IGFBP-3, by RIA, increased from 3.2 to 7.0 μg/ml and mean ALS from 18.4 to 41.6 μg/ml, while mean IGFBP-1 decreased slightly from 497 to 428 ng/ml. Following transplantation, mean IGFBP-3 decreased to 4.8 μg/ml, however mean ALS remained elevated at 45.2 μg/ml. Mean IGFBP-1 fell further to 97.2 ng/ml. This study shows that rhGH treatment in CRF results in significant increases in serum levels, by RIA, of IGFBP-3 and of ALS, but not of IGFBP-1. There appear to be forms of IGFBPs present in whole serum which do not bind well to the radio-ligand during WLB, and immunoblotting may be necessary to supplement this technique for IGFBP identification. The reduction in IGFBPs following transplantation suggests correction of impaired clearance by the diseased kidney.

A naturally occurring xenoantibody, anti-Gal, recognises epitopes in pig kidney, heart and liver and is functionally active: Relevance to xenotransplantation. *M. Tange, A.M. Fournier, I.E. Birchall, M. Romanella, A. Aminian, A.G. Kyriazis, M.J. Pearse, W.R. Adam, and A.J.F. d'Apice, Dept. of Clinical Immunology, St. Vincent's Hospital, Fiztroy, Melbourne, Australia, 3065 and Dept. of Pathology Univ. of Melbourne, Dept. of Renal Medicine, Repatriation Hospital, Heidelberg.* Human naturally occurring antibodies against xenoantigens are the primary effective cause of hyperacute rejection. There is anti-galactosyl galactose antibody (anti-Gal) specific for Gal alpha 1,3 Gal beta 1,4 GlcNAc in humans but not in pigs due to the absence of the enzyme alpha 1,3 galactosyl transferase in humans. We have found Gal epitopes in pig renal cortex, heart and liver by indirect immunofluorescence and immunoperoxidase staining. Anti-Gal strongly stained endothelial cells of glomerular capillaries, arteries, and veins in porcine organs. The specificity of binding was demonstrated by inhibition with terminal alpha 1 galactosyl carbohydrates such as melibiose (Gal alpha 1,6 Glc) which reduced the staining to near background. The functional role of anti-Gal was assessed. Porcine aortic endothelial cells, labelled with ^{51}Cr, in the presence of rabbit complement, were lysed by heat inactivated normal human serum, or by the anti-Gal component of human serum purified by affinity chromatography on a galactosyl-galactose (Synsorb 115) affinity column. The IgM fraction of anti-Gal was responsible for lysis. Agglutination of 0.5% pig erythrocytes

by pooled normal human AB serum can be inhibited by terminal alpha 1 galactosyl sugars. Human serum induced lysis of pig aortic endothelial cells can also be inhibited only by terminal alpha 1 galactosyl sugars. In an *ex vivo* model of hyperacute rejection, when a rat heart is perfused with normal human plasma there is prolongation of time to hyperacute rejection when terminal alpha 1 galactosyl sugars are perfused with the plasma. In conclusion, Anti-Gal binds to alpha 1 Gal epitopes on porcine endothelium, has a functional role in an *ex vivo* model of xenograft rejection and is inhibitable by specific sugars.

Increased expression of transforming growth factor β_1 (TGFβ_1) in a model of chronic renal allograft rejection. *A.N. Stein-Oakley, A. Tzanidis, P.J. Fuller, P. Jablonski, and N.M. Thomson, Department of Medicine, Monash Medical School, Alfred Hospital, Prahran, 3181; Monash Department of Surgery, MMC; Prince Henry's Institute of Medical Research; Melbourne, Australia.* TGFβ_1 has widespread effects on extracellular matrix (ECM) and modulates proliferation of many cell populations including mesangial cells and fibroblasts. TGFβ_1 stimulates its own production as well as that of other cytokines such as PDGF and IL-1. Chronic rejection (CR) is responsible for the loss of 50% of the renal grafts after the first year. The deposition of collagen and other ECM components and the proliferation of fibroblasts, resulting in damage characteristic of CR, are consistent with a possible involvement of TGFβ_1. This study describes the expression of TGFβ_1 in a rat model of CR. Studies were performed on transplanted kidneys from tolerised AS recipients of DA allografts at 5 days ($N = 3$), 1 ($N = 5$) and 3 ($N = 9$) months post transplantation. Grafts at 1 and 3 months demonstrated progressively more severe features of CR. Controls were kidney samples from 4 normal rats (NRK), 6 isograft recipients (ISO) and 4 uninephrectomized (U) rats killed 3 months post surgery. TGFβ_1 gene expression was evaluated by Northern blot analysis of total RNA using a rat TGFβ_1 cDNA probe. RNA levels were standardised against GAPDH expression. Densitometric analysis revealed 3.8, 4.2 and 2.6 fold increases in TGFβ_1 expression in the CR model at 5d, 1 ($P < 0.05$) and 3 ($P < 0.01$) months respectively versus NRK. Increased TGFβ_1 expression in CR suggests it may be involved in the development of chronic damage. Both transcripts of TGFβ_1 mRNA (2.5 and 1.9 kb) were expressed in the CR model. The ratio of the 2.5 kb/1.9 kb transcripts was lower in the CR model [5.4 ± 0.5 (mean ± SEM) and 4.6 ± 0.6 ($P < 0.05$) at 1 and 3 months respectively] than in NRK (8.9 ± 1.2). This is consistent with previous findings in infarcted rat heart suggesting that the regulation of the 1.9 kb transcript may be related to injury. TGFβ_1 expression in U kidneys did not differ from NRK. Surprisingly, TGFβ_1 expression in ISO was increased 3.9-fold ($P < 0.05$) versus NRK although ISO showed only minor chronic changes. Thus increased TGFβ_1 expression may represent a response to transplant injury. However, there could be differences in the activation state of TGFβ_1 (latent or activated) in CR versus ISO, or a different relative balance of TGFβ_1 and other cytokines in the tissue.

Enalapril controls post-transplant erythrocytosis by suppression of erythropoietin production. *K.C. Wong, N. Bandler, P.G. Kerr, and R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton Vic 3168.* Erythrocytosis is a common finding in renal transplantation. The aetiology may be multifactorial; including an increased production of Erythropoietin (EPO). Clinical studies using Angiotensin Converting Enzyme (ACE) inhibitors to treat post-transplant erythrocytosis (PTE) have predictably demonstrated its haematocrit (Hct) lowering effect but the data on changes in EPO levels are inconsistent. We therefore studied the effect of ACE inhibition on serum EPO levels and its potential benefit in controlling PTE. We treated 15 renal transplant patients (14 M/1 F), mean age 51.1 ± 11.6 (range 23–64 yrs) who had a persistent Hct of 51% or greater with Enalapril. Twelve patients had venesection before enrolling in the study. Baseline measurements of Hct, creatinine clearance and serum EPO levels were made before Enalapril therapy. Enalapril was started at 2.5 to 5 mg daily and the dose was adjusted as necessary for blood pressure control. The patients were followed at 1–2 monthly intervals with repeat Hct, creatinine clearance and serum EPO measurements. The mean Hct fell from 52.8 ± 1.7 to 43.7 ± 3.4 ($P = 0.001$) and serum EPO fell from 24.1 ± 21.0 to 7.5 ± 6.8 mU/ml ($P = 0.018$) after 2 to 6 months of Enalapril therapy. The mean blood pressure, serum potassium and creatinine clearance did not change significantly during follow up. It is concluded that Enalapril is safe and effective in controlling PTE by suppression of EPO production.

Hyperlipidaemia and the response to simvastatin in renal transplant recipients. *C.S. Ong, C.A. Pollock, R.J. Caterson, D.A. Waugh, J.F. Mahony, and L.S. Ibels, Department of Renal Medicine Royal North Shore Hospital, St Leonards, 2065, Australia.* A seven year prospective study assessing the lipid profiles of 192 patients with functioning renal transplants, their aetiological associations and response to therapy, in particular simvastatin, was undertaken. The files of patients entered into the study were also reviewed for retrospective data regarding their lipid profile. The mean time from transplantation to last follow up was 103 ± 4.8 (range 1–240) months. 7.8% had diabetic nephropathy as a primary diagnosis. A significant rise in serum cholesterol occurred 1–3 months after successful engraftment, stabilising over the next 3 years, at which time hypercholesterolaemia was present in 71.3% of patients. There were independent associations of serum cholesterol with prednisone dosage ($P < 0.05$), renal dysfunction ($P < 0.05$) and smoking ($P < 0.05$) in the early post transplant period. Those patients whose immunosuppression included cyclosporin had lower serum cholesterol levels than those receiving azathioprine and prednisone ($P < 0.02$). The presence of diabetes mellitus, hypertension or the form and duration of prior dialysis did not independently influence the lipid profiles. During the period of study 22 patients (11.5%) died, 54.5% due to vascular causes. Those who died a vascular death had higher serum cholesterol levels than those who died of other causes, which reached statistical significance at 3 years post transplant (7.7 ± 0.4 vs. 5.5 ± 0.5 mmol/l; $P < 0.02$). No differences in the HDL subfraction nor in serum triglycerides were observed in those who died of vascular causes. Cholestyramine was introduced in 30 patients, only 2 of whom continued with therapy beyond 3 months. Simvastatin was used in 43 patients, 20 of whom were receiving cyclosporin, resulting in a reduction in serum cholesterol of 16.5%, from 7.0 ± 0.2 to 5.7 ± 0.1 mmol/l ($P < 0.001$) and in serum triglycerides of 21%, from 2.9 ± 0.4 to 2.3 ± 0.3 mmol/l ($P < 0.05$). No evidence of muscle, liver or renal toxicity occurred in 15.4 ± 0.9 months of follow up. We conclude that lipid abnormalities contribute to the high vascular mortality in renal transplant recipients and simvastatin is a safe and effective means of improving the lipid profile.

The role of eosinophils in acute renal allograft rejection. *Wang Hongwei, R.S. Nanra, Anne Stein, Leanne Avis, Anna Price, and A.D. Hibberd, Nephrology, Transplantation and Anatomical Pathology Units, John Hunter Hospital, Newcastle, NSW, 2305.* Tissue eosinophils (EOSs) have been previously implicated in allograft rejection and graft loss. A retrospective cohort study was undertaken to evaluate the role of EOSs in acute renal allograft rejection. Tissue EOS density was measured morphometrically. Data from 71 patients with 114 renal biopsies with acute allograft rejection were compared with that from 26 controls. The median tissue EOS density (0.4–1.1 EOSs/$\mu m^2 \times 10^6$ and the median peripheral blood EOS (1.5–3.0%) in all grades of acute interstitial rejection and in acute vascular rejection were higher than that in controls (0.0 EOSs/$\mu m^2 \times 10^6$, $P < 0.0023$, and 0.9%, $P < 0.035$, respectively). In all grades of rejection, 36–54% of biopsies had tissue EOS density ≥1 EOS/$\mu m^2 \times 10^6$, and 20–36% of patients had peripheral blood EOS ≥4%, compared to 0% and 4%, respectively, in controls ($P < 0.000$ and $P = 0.0245$). The sensitivity, specificity and overall accuracy of predicting acute rejection with tissue EOS density ≥1 EOS/$\mu m^2 \times 10^6$ is 41%, 100% and 52%, and for peripheral blood EOS ≥4% is 23%, 96% and 40%, respectively. The median tissue EOS density in acute rejection with graft loss was 1.9 EOSs/$\mu m^2 \times 10^6$ compared to 0.2 EOSs/$\mu m^2 \times 10^6$ in acute rejection without graft loss ($P = 0.014$), and 67% of acute rejection with graft loss had tissue EOS density ≥1 EOS/$\mu m^2 \times 10^6$ compared to 28% of acute rejection without graft loss ($P = 0.028$). It is concluded that (1) peripheral blood EOS and increased tissue EOS density occur in up to 50% of patients with acute renal allograft rejection, (2) these may be regarded as diagnostic and prognostic markers of acute rejection, and (3) the eosinophilic response may represent a secondary or nonclassical pathway of allograft damage.

The association of acute leukaemia with the use of chlorambucil following renal transplantation. *W.K.W. Ho, M.R. Robertson, G.J. Macdonald, J.A. Charlesworth, and B.A. Pussell, Dept. of Nephrology, Prince Henry Hospital and University of New South Wales, Sydney, NSW 2036 Australia.* An increased incidence of neoplasms has been recognised following renal transplantation. Although these have been predominantly squamous cell carcinomas of skin, lymphomas and some mesenchymal

tumours have been reported. Acute leukaemia has been reported following the successful treatment of Hodgkin's disease and breast cancer, especially with the use of alkylating agents. Because of these findings and the recent reports of the use of chlorambucil in the treatment of membranous glomerulonephritis we analysed, retrospectively, the use of different immunosuppressive agents post renal transplantation in our unit from 1.1.65 to 31.12.88. Data was obtained from the hospital records and the Australian and New Zealand Dialysis and Transplant Registry. Three hundred forty-eight patients on treatment for more than three months were assessed. Two hundred twenty-three received azathioprine alone, 90 cyclosporin A ± azathioprine and 35 chlorambucil. All received corticosteroids and 92 received anti-lymphocyte preparations in addition to the other agents. A total of 12 patients developed leukaemia. None of these had received immunosuppressive drugs pre-transplant and all but one was a primary renal graft. The type of leukaemia in each case was acute non-lymphocytic and all developed the leukaemia after at least 11 months of treatment. Nine patients had been treated with chlorambucil, 2 with azathioprine only and 1 with cyclosporin A ± azathioprine. The association between chlorambucil exposure and development of leukaemia was highly significant ($\chi^2 = 52.93$; $P < 0.0001$). In addition, within the chlorambucil treated patients there was a relationship between total dose of chlorambucil and development of leukaemia (mean 5537 mg versus 3138 mg; $t = -2.59$; $P < 0.02$). Of 71 patients who received anti-thymocyte preparations, 2 developed leukaemia and these 2 had also received chlorambucil. None of 21 patients treated with OKT3 developed leukaemia. The time from transplant to diagnosis of leukaemia ranged from 11–164 months (median 70) and from diagnosis to death was 8–259 days (148). All patients died from the leukaemia. This highly significant association between the use of chlorambucil and subsequent development of acute leukaemia suggests caution in the use of this agent in renal transplantation and may also have implications for the recent interest in its use to treat diseases such as membranous glomerulonephritis.

Do ACE inhibitors prevent diabetic nephropathy by reducing glomerular protein synthesis? *M.M. Makarious, K.A. Duggan, G.J. Macdonald, and J.A. Charlesworth, Dept. of Nephrology, Prince Henry Hospital, Sydney, NSW 2036, Australia.* Systemic and glomerular hypertension are implicated in the initiation and progression of nephropathy in clinical and experimental diabetes. The choice of antihypertensive agent is controversial. Angiotensin converting enzyme (ACE) inhibitors may confer benefits in reducing albuminuria in excess of the degree of blood pressure control, possibly by modulating the intrarenal renin angiotensin system. We studied the effects of similar blood pressure control using enalapril (10 mg/kg/day) or verapamil (13 mg/kg/day) on glomerular angiotensin II receptors, glomerular protein, creatinine clearance and proteinuria in the spontaneously hypertensive rat (SHR) three months after induction of diabetes by streptozotocin. Blood glucose was maintained at 8–10 mmol/l by daily insulin injection and blood pressure at 105–120 mm Hg systolic in both groups. Creatinine clearance was lower in enalapril (0.46 ± 0.05 ml/min; $P < 0.001$) and verapamil (0.55 ± 0.06 ml/min; $P < 0.03$) groups than control (0.77 ± 0.06 ml/min) while proteinuria was lower in the enalapril group (E: 5.6 ± 0.79, C: 11.4 ± 1.50, V: 13.2 ± 1.47 mg/24 hr; E vs. C: $P < 0.0025$, E vs. V: $P < 0.001$). Glomerular Ang II receptors were decreased in the verapamil group (1804 ± 202 fmoles/mg protein) compared with enalapril (3079 ± 149, $P < 0.001$) and control (2763 ± 345, $P < 0.01$). Glomerular protein was least in the enalapril group (48 ± 3.1 ng/glomerulus) and greatest in the verapamil group (84 ± 9.8 ng/glomerulus, $P < 0.0025$). We conclude that both antihypertensive regimes are equally effective in lowering blood pressure and preventing hyperfiltration. However, treatment with ACE inhibitors significantly reduces proteinuria. In addition, by reducing Ang II stimulated glomerular protein synthesis they may play a role in preventing glomerular sclerosis.

The effect of intravenous ouabain on pressor responsiveness in healthy volunteers. *G.B. Pidgeon, A.M. Richards, M.G. Nicholls, R.R. Bailey, K.L. Lynn, L.K. Lewis, and T.G. Yandle, Departments of Nephrology, Cardiology and Endocrinology, Christchurch Hospital, Christchurch, New Zealand.* To assess the effects of ouabain on pressor and vasoactive hormone responsiveness, 10 healthy volunteers were pre-treated with ouabain (0.5 mg i.v. 42 and 18 hrs before study) or placebo, prior to pressor challenge with angiotensin II (Ang II) (2, 4 and 8 ng/kg/min for 30 minutes per dose) and noradrenaline (NA) (5, 15 and 45 ng/kg/min for 15 minutes per dose) in single-blind, randomised, placebo-controlled, cross-over studies. Blood pressure and heart rate (HR) were recorded manually at 15-min (Ang II) and 5-min (NA) intervals. Holter ECG monitoring was performed. Vasoactive hormones were measured at baseline and during infusions. There were no differences at baseline between the two study days regarding plasma electrolytes, creatinine, mean arterial pressure (MAP) or HR. Baseline pulse pressure, however, was significantly greater following ouabain (47 ± 4 mm Hg vs. 41 ± 1 mm Hg) [mean ± SEM] ($P < 0.05$). The mean maximum increments in MAP during Ang II and NA infusions were 17.5 ± 1.1 and 10.5 ± 1.3 mm Hg respectively following ouabain, and 19.2 ± 1.3 and 10.4 ± 1.5 mm Hg following placebo (NS). The mean HR was lower during both infusion periods on the ouabain study day compared to control ($P < 0.05$). Baroreceptor reflex sensitivity was unchanged. Baseline plasma levels of Ang II, aldosterone, plasma renin activity, atrial and brain natriuretic peptide, cyclic GMP, NA and adrenaline, and achieved levels during the two infusions, were similar on the two study days. We conclude that short-term ouabain administration does not alter pressor responsiveness or plasma levels of vasoactive hormones in healthy volunteers.

The role of C-type natriuretic peptide in control of renal function in the sheep. *Margaret B. Fraenkel, G.P. Aldred, N.A. Yates and J.G. McDougall, Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria, 3052.* Three receptors for the atrial natriuretic peptide family have been identified. The GC-B receptor is a membrane bound guanylate cyclase receptor which appears to have C-type natriuretic peptide (CNP) as its natural ligand. CNP was initially thought to be confined to the central nervous system, but mRNA for both CNP and GC-B has recently been identified in most body tissues, where they are thought to be possibly involved in a vascular autocrine or paracrine system. As a CNP bolus had been reported to cause an antinatriuresis, without change in blood pressure[1], we wished to see if mRNA levels for the GC-B receptor varied with sodium status. A 461 bp fragment of cDNA for the ovine GC-B receptor was cloned from ovine pituitary RNA using reverse transcriptase-PCR. There was 90% nucleotide sequence identity to the human GC-B receptor and 97% similarity at the amino acid level. The cDNA fragment was used as a probe for northern analysis of mRNA isolated separately from renal cortex and medulla of 6 Na loaded, 6 Na depleted and 4 control sheep. Levels of mRNA for the receptors were quantitated using laser densitometry and standardised to β-actin expression. The level of mRNA for the GC-B receptor in the renal cortex was significantly higher in sodium depleted animals (1.57 ± 0.17, mean ± SEM) than in sodium loaded (0.86 ± 0.12) or control (0.84 ± 0.18) animals ($P < 0.05$). To investigate the role of CNP in renal function in sheep, CNP was infused into the renal artery of 4 uninephrectomised, conscious, sodium replete merino ewes at a rate of 185 μg/hr for one hour. MAP fell from 80 ± 3 mm Hg during the control period to 72 ± 2 mm Hg in the 15 min period following the infusion ($P < 0.01$). There was no significant effect on GFR, ERPF, urine flow rate, Na, K or Ca excretion, although the animals responded to ANP (50 μg/hr) with the expected increase in Na excretion (from 153 ± 33 μmol/min to a maximum of 456 ± 43 μmol/min). In summary, we have shown that mRNA for the GC-B receptor is present in sheep kidney, and levels increase approximately two-fold with Na depletion. However we have been unable to detect any effect of CNP, its proposed natural ligand, on renal function.

1. STINGO et. al.: (1992) Amer. J. Physiol. 262:H308–312

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Expression and molecular characterisation of tissue factor in crescentic glomerulonephritis. *J.H. Erlich, P.G. Tipping, J.A. Apostolopoulos, N. Mackman, D.J. Luskotoff, and S.R. Holdsworth, Department of Medicine Monash University, Clayton, Vic 3168 and Scripps Research Institute, La Jolla, CA, USA.* Augmented pro-coagulant activity (PCA) with the characteristics of tissue factor (TF) has been demonstrated in a rabbit model of crescentic glomerulonephritis (GN) in association with proteinuria, prominent fibrin deposition, and macrophage influx. TF is currently thought to be the major *in vivo* activator of coagulation. A membrane glycoprotein with several N-linked glycosylation sites, TF is dependent on the presence of phospholipids for activity. To confirm that glomerular PCA was due to TF, a monoclonal anti-rabbit TF antibody was prepared that totally inhibited glomerular PCA. TF expression was assessed at days 1, 4, 7, and 12 in a preimmunised rabbit model of anti-GBM crescentic GN and in normal rabbits. TF functional activity was determined in a one stage

prothrombin assay. TF molecular weight was determined by Western blotting and subsequent immunodetection with a monoclonal antibody to rabbit TF. TF mRNA was assessed by Northern blotting and *in situ* hybridization with a cDNA to rabbit TF. TF activity was upregulated at day 1 (208 ± 70 au ($P < 0.05$)) cf normal (40 ± 1670 au) and remained elevated throughout. Glomerular TF antigen was not increased until day 4 (2.43 ± 0.09 au ($P < 0.045$)) cf normal (1.69 ± 0.24 au). Rabbit glomerular TF antigen exhibited a higher molecular weight (Mw) than standard rabbit brain TF. The Mw of glomerular TF on days 4, 7, and 12 of this disease (the days associated with prominent glomerular macrophage infiltration) showed higher Mw bands to those seen in normal glomeruli. The Mw of glomerular TF on these days corresponds to that of TF expressed by activated macrophages. The variation in TF Mw on different days and between organs was shown to be due to variable glycosylation. TF mRNA was demonstrated to be intraglomerular by *in situ* hybridisation. Glomerular TF mRNA was not significantly increased until day 4 (169.8 ± 18.3 AU ($P < 0.044$)) cf normal (100 ± 12.1 AU). In this model of GN increased glomerular PCA is due to TF; increased TF functional activity precedes increases in TF antigen and mRNA; and the higher Mw of TF at day 4 is associated with macrophage influx. This suggests macrophages are the most likely source of this Mw TF. The dissociation of the increase in TF functional activity and antigen expression may be due to a change in the TF phospholipid association and/or the presence of inhibitors. Alteration in TF glycosylation may in turn alter its avidity for inhibitors.

Risk factors for acute pyelonephritis in preschool children. *J.C. Craig, L.P. Roy, and J.F. Knight, Department of Nephrology, Royal Alexandra Hospital for Children, Sydney, NSW, 2050.* To determine the epidemiology, microbiology, and natural history of the first symptomatic urinary tract infection (UTI) in preschool children with no known predisposing condition, a prospective study of all eligible children presenting acutely to this hospital has been established, and the frequency of acute pyelonephritis (APN) and risk factors for this precursor of renal scarring have been determined. To date, 125 children have been identified, primarily by analysis of all urine samples sent to the microbiology department for microscopy and culture. Patients were reviewed whilst in the acute phase of the illness and all relevant clinical and microbiologic details were recorded. The diagnosis of APN was made by dimercaptosuccinic acid scan (DMSA). Abnormalities in the DMSA scan were graded from 1–4 (grade 1: 1 or 2 focal defects, grade 2: 3 or more focal defects, grade 3: diffuse parenchymal abnormality >10% differential function, grade 4: 10% differential function), with the DMSA score defined as the sum of the score of each of the 2 renal units. Of the 125 children with a proven UTI presenting to this hospital, 121 (97%) have had a DMSA scan from 1–25 days after diagnosis (mean 7.7 days, SD 4.4 days). Thirty-four percent had scan evidence of APN (41 patients, 52 renal units). One hundred twenty-two patients (98%) had a renal ultrasound (USS) examination. Vesicoureteric reflux (VUR) was present in 31% of 106 patients in whom a micturating cystourethrogram (MCU) has been performed. VUR was detected in only 59% of children with APN. The USS correctly detected APN in 20%. All variables of epidemiology, clinical presentation, microbiology, ultrasound and MCU results were analysed as possible risk factors for acute pyelonephritis (abnormal DMSA scan) using linear regression. Only a history of previous unexplained fever (relative risk of 4.3 (95% CI 1.2–15.4, $P = 0.027$) and VUR (relative risk of 5.6 (95% CI 2.2–15.4, $P = 0.0001$) were found to be significant predictors of APN. These same variables were analysed by logistic regression to determine independent predictors of the DMSA score. VUR, intrarenal reflux (IRR), and calyceal dilatation on ultrasound were all found to be highly predictive of the DMSA score. We conclude that a history of unexplained fever and VUR predict for APN, and VUR, IRR and calyceal dilatation predict for the severity of APN. However, the sensitivity of the MCU in detecting preschool children with APN is only 59%, therefore this test alone, or in combination with the USS, does not detect acute parenchymal defects in 40% who may be at risk of permanent scarring.

Peritoneal dialysis solutions (PDS) inhibit human mesothelial cell biosynthesis *in vitro*. *S.D. Bird, F.M. Munro, and R.J. Walker, Medicine Dept., Otago Medical School, Dunedin, NZ.* Peritoneal dialysis solutions (PDS) utilise high glucose concentrations (76–214 mM) for ultrafiltration. Recent studies have suggested that PDS may be cytotoxic to mesothelial cells. The initial peritoneal mesothelial cell (PMC) response to exposure to PDS has not been well defined. Aims: to define the biosynthetic response of human PMCs to PDS compared to an epithelial cell line (LLC-PK). Human PMCs were obtained from omentum by enzymatic digestion and grown in DME/F-12 media with 10% FCS. Cells were used at passage 2. Confluent cells in 24 well plates were exposed to KR buffer (glucose 5.5 mM), 1.5% (76 mM), 2.5% (126 mM), 4.25% (214 mM) PDS for 2–8 hours. DNA and protein synthesis was measured by ^{3}H-thymidine (^{3}HT) and ^{3}H-leucine (^{3}HL) incorporation (cpm/mg protein). Cell viability was measured by trypan blue exclusion. Results are mean ± SEM $N = 10–20$. $^*P < 0.05$.

T	Pig (LLC-PK$_1$) Kidney Cells ^{3}HT Incorporated Glucose (mM)			
h	5.5	76	126	214
Control				
2	62.8 ± 3.5	56.9 ± 5.6	58.9 ± 8.0	43.0 ± 5.6*
4	32.7 ± 2.5	22.5 ± 4.0*	24.8 ± 4.7	5.9 ± 1.9*
6	25.1 ± 0.8	23.0 ± 1.6*	8.2 ± 2.1	1.4 ± 0.4*
8	44.2 ± 5.4	10.6 ± 1.7*	6.1 ± 1.2*	1.1 ± 0.2*

	Human PMC ^{3}HT Incorporated Glucose (mM)		
5.5	76	126	214
Control			
45.6 ± 5.7	20.5 ± 3.7*	15.4 ± 2.8*	11.8 ± 2.1*
91.2 ± 8.5	40.1 ± 4.2*	35.5 ± 3.3*	40.1 ± 12.0
51.0 ± 6.5	28.4 ± 3.2*	34.2 ± 5.7*	27.1 ± 7.1*
28.1 ± 3.9	10.7 ± 2.3	10.1 ± 1.4*	13.4 ± 3.6*

PDS were toxic to both cell lines with a significant reduction in viable cells by 6 hours. DNA synthesis was significantly reduced as early as 2 hours in PMC at all concentrations. Protein synthesis was similarly inhibited. PMC were much slower to recover following re-exposure to normal culture media. This is most likely an acute response to the high glucose concentrations. Experiments are currently underway to exclude pH or osmotic effects. This would suggest alternative agents to glucose in PDS need to be found.

Circulating renotropic factors and epithelial transport. *C.A. Pollock, M.S. Nobes, A.Z. Györy, P.T. Heng, and M.J. Field, Department of Medicine, University of Sydney, Royal North Shore Hospital, St Leonards, 2065, Australia.* It has been postulated that circulating growth factors play a role in the development of renal hypertrophy. In order to investigate the effect of these factors on glomerular filtration and epithelial transport, micropuncture experiments were performed on normal 6–7 week old Sprague Dawley rats infused with 20% plasma derived from rats in whom nephrectomy had been performed 3 days previously; $N = 4$. Animals infused with plasma from rats which had undergone sham nephrectomy served as controls; $N = 4$. When animals infused with plasma from nephrectomised animals (NxP) were compared with those infused with control plasma (CP), the former had a higher tubular fluid flow rate measured at both the late proximal (LP) (26.7 ± 1.6 vs. 18.3 ± 1.4 nl/min; $P < 0.0005$) and early distal (ED) (14.9 ± 1.0 vs. 7.8 ± 1.0 nl/min; $P < 0.0001$) site, which was reflected in the final urine flow rate (16.1 ± 3.4 vs. 5.1 ± 0.8 μl/min; $P < 0.005$). The single nephron glomerular filtration rate (SNGFR) was higher as determined at the LP (45.8 ± 2.8 vs. 35.7 ± 2.3 nl/min; $P < 0.01$) and ED (34.5 ± 2.5 vs. 28.1 ± 1.8 nl/min; $P = 0.05$) site. However, this increase was not reflected in the whole kidney GFR (1.04 ± 0.06 vs. 0.89 ± 0.06 mls/min/100 g; $P = 0.07$), suggestive of a preferential increase in filtration in the outer cortical nephrons. Tubular Na transport was higher in the animals infused with NxP, as evidenced by a decrease in the fractional delivery of Na at the early distal site (4.5 ± 0.4 vs. 6.5 ± 0.4; $P < 0.02$). However, in the final urine there was a significant increase in the fractional sodium excretion in animals infused with Nx plasma (0.67 ± 0.14 vs. 0.29 ± 0.09%; $P < 0.05$) indicating that natriuresis and probably diuresis was a result of inhibition of Na and water transport in the late distal tubule and collecting duct. In conclusion these experiments demonstrate that circulating factors induced by a reduction in renal mass

significantly alter glomerular filtration and tubular Na transport. Although the present experiments do not define the nature of these factors, it is likely that they play a role in the subsequent development of compensatory renal hypertrophy.

Detection of hydroxyl radicals following ischaemia and reperfusion in isolated rat kidneys. *M. Kadkhodaee, Z.H. Endre, R.A. Towner, and M. Cross, Department of Medicine, University of Queensland, Royal Brisbane Hospital, Qld 4029.* The role of oxygen derived free radicals in acute renal failure remains controversial. In order to quantitate hydroxyl radical generation *in vitro* in isolated rat kidneys during hypoxia and during reperfusion following ischaemia, salicylate was used as a chemical trap to react with hydroxyl radicals to produce 2,5-dihydroxybenzoic acid (DHBA) which could be detected by HPLC combined with electrochemical detection (ED). Right kidneys from male Wistar rats perfused with Krebs-Henseleit buffer (KHB) with 6.7% albumin. Perfusion pressure, flow and oxygen tension were continuously monitored. Function was monitored from the inulin clearance and sodium and potassium excretion. Five groups (each $N = 5$) were studied: group 1 (normal control), perfused for 75 min; group 2 (salicylate control), supplemented with 0.5 mM salicylate; group 3 (ischaemia), salicylate plus ischaemia induced for 15 min followed by 15 min reperfusion; group 4 (DMTU-ischaemia), 15 mM DMTU plus salicylate, before induction of ischaemia followed by reperfusion; group 5 (hypoxia), salicylate followed by 15 min of hypoxic perfusion. Under normoxic conditions in salicylate control kidneys (group 2), peaks corresponding to the hydroxyl radical were detected as 2,5-DHBA. During ischaemia and reperfusion (group 3) there was more than a two fold increase in production of hydroxyl radical ($P < 0.001$). Adding 15 mM DMTU before ischaemia (group 4) prevented the increase in 2,5-DHBA. Induction of 15 min of hypoxia (without normoxic reperfusion) impaired kidney function predictably (group 5) but did not increase 2,5-DHBA. This supports the recent observation that DMTU and DMSO protect against hypoxic injury by a mechanism independent of the binding of free radicals. The increase of 2,5-DHBA in ischaemic-reperfused kidneys, indicates hydroxyl radical formation which is supported by the observation that pretreatment with DMTU prevented any increase in 2,5-DHBA. This is the first observation *in vitro* or *in vivo* of specific hydroxyl radical generation following ischaemia-reperfusion in the intact kidney.

1. Cross, M., Endre, Z.H., Stewart-Richardson, P. Cowin, G.J., et al.: *Magn. Reson. Med.* 30:465–475, 1993.

Proximal tubular (PT) amphotericin B (AMB) toxicity: An electron microprobe study. *C.A. Pollock, M. Dyne, M.J. Field, N. Salipan Moore, S. Reddy, D. Cockayne, and A.Z. Györy, Department of Medicine and Electron Microscope Unit, University of Sydney, Royal North Shore Hospital, St Leonards, NSW, 2065.* The renal toxicity of AMB clinically manifests largely as distal tubular injury, with K wasting, renal tubular acidosis and a reduction in GFR. The defect is presumed to be due to an increase in permeability of Na, K and H ions in the tubular epithelium. As this toxicity has been shown *in vitro* to uniformly affect the nephron, it is surprising that more marked proximal tubular dysfunction is not observed. The following experiments, on male Wistar rats, were designed to assess using electron microprobe analysis (EMPX), the alterations in intracellular electrolyte concentrations in proximal tubular cells as a result of AMB treatment. Five animals were prepared as for micropuncture experiments and infused with modified Ringers solution at 1.2 ml/100 g/hr. After equilibration, two 30 minute clearance collections were done. AMB was then infused at a rate of 1 mg/kg/hr and after equilibration 2 further clearance collections were made. At the conclusion of the clearance study isotonic RbCl was infused at 0.5 mmol/kg over 30 secs, as a further marker of K transport, and the kidney immediately removed. Controls were animals infused with Ringers solution or AMB buffer alone. Clearance data confirmed a drop in GFR in the AMB treated rats compared to control (0.69 ± 0.11 vs. 1.27 ± 0.08; $P < 0.001$). However, no difference in the urine flow or fractional excretion of Na and K was evident. Measurement of intracellular electrolytes of PT cells in mmol/kg wet weight by EMP analysis demonstrated no difference in Na (16.1 ± 0.6 vs. 17.2 ± 0.5) or K (129.7 ± 2.1 vs. 131.3 ± 2.0). However, a significant reduction in Cl occurred (12.7 ± 0.4 vs. 14.1 ± 0.4; $P < 0.05$). Intracellular Rb accumulation was significantly reduced in the AMB treated animals (3.9 ± 0.4 vs. 6.6 ± 0.4; $P < 0.0001$) suggestive of a reduction in basolateral Na-K ATPase activity. These results do not support the tenet that AMB causes a generalised increase in epithelial cell membrane permeability, nor direct cellular toxicity in the doses studied. Rather they suggest that a primary reduction in GFR results in a load-dependent decrease in proximal tubular Na transport.

Alterations in plasma ANP, BNP and cardiac volumes with changes in intravascular volume in chronic renal failure. *J.M. Corboy, R.J. Walker, M. Simmonds, G.T. Wilkins, A.M. Richards, and E.A. Espiner, Medicine Dept., University of Otago Medical School, Dunedin, NZ.* Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels are elevated in conditions of fluid overload including chronic renal failure (CRF). This study was designed to investigate the changes in plasma ANP and BNP with changes in atrial and ventricular volumes following alterations in intravascular volume during hemodialysis. Eight patients (6 males) with chronic renal failure (CRF) on haemodialysis, who were normotensive with no heart disease were studied. Two phases in the study. 1. Ultrafiltration (UF) (3% body weight) over 2 hours. 2. Volume repletion over 2 hours plus an additional 0.5l saline. Weight (bed scales), blood pressure, heart rate were recorded and venous samples (electrolytes and hormones) were measured every 30 mins. Two-dimensional echocardiography was performed at 0, 120, 240, 270 mins and chamber volumes calculated. Results: Changes in plasma ANP during UF correlated significantly with changes in LAV ($r = 0.64$). There was no significant correlation between BNP and cardiac volumes during UF or volume replacement.

Time (min)	Wgt (Kg)	ANP (pmol/l)	BNP (pmol/l)	LAV cm^3	LVV cm^3
0	77.4 ± 14	46.3 ± 7.5	21.5 ± 4.5	51 ± 4	171 ± 16
120	75.0 ± 13*	26.5 ± 4.7*	19.1 ± 3.9*	39 ± 4*	131 ± 16*
240	77.2 ± 14	59.5 ± 16.8	20.4 ± 4.2	51 ± 4	164 ± 17
270	77.9 ± 14	71.0 ± 20.8#	20.8 ± 4.3	57 ± 6	198 ± 30

* $P < 0.05$, # $P < 0.05$ vs. time 0

Discussion. Baseline levels of ANP and BNP were elevated above normal with a significant reduction in plasma ANP and BNP (minor) following UF. The exaggerated release of ANP during volume repletion was not paralleled by changes in BNP levels. Changes in BNP levels were small with no correlation with atrial or ventricular volumes. This would suggest that in CRF, ANP release is a more important factor in regulating acute changes in intravascular volume. In CRF and normal myocardial function, BNP appears to have only a minimal role in acute changes in intravascular volume, despite elevate basal levels.

***In vivo* regional image-guided ^{1}H-NMR spectroscopy of rat kidney.** *R.A. Towner, Z.H. Endre, S. Wilson, and D. Doddrell, The University of Queensland, Royal Brisbane Hospital and Centre for Magnetic Resonance, Brisbane, Qld. 4072 Australia.* We present the first noninvasive study of *in vivo* cortical and medullary image-guided proton NMR spectroscopy in rat kidney. This technique was also used to monitor biochemical changes during and after *in vivo* ischaemia-reperfusion injury. Image-guided, microvolume, high resolution localized ^{1}H-NMR spectra were obtained *in vivo* at 4.2 T in kidneys of 150 g Wistar rats from 14–20 μl volumes (2.2 × 2.2 × 2.2 mm^3 to 2.7 × 2.7 × 2.7 mm^3) using the VOSY pulse sequence with CHESS water suppression, an actively decoupled resonator/surface coil assembly, a high strength (30 Gauss/cm) gradient set, and respiratory gating. Spatial coordinates for the localized ^{1}H-NMR spectra were obtained from transverse ^{1}H-NMR images (spin echo; TR/TE 18/800 ms) of anaesthetized rats (1.5% Isoflurane). The obtained microvolume spectra allowed non-invasive *in vivo* differentiation of biochemical information between cortex and medulla. The main biochemical differences detected were much higher amounts of osmolytes, such as the trimethylamines (TMAs; e.g., betaine), inositol and sorbitol in the renal medulla than in the renal cortex. The same sequences were used to monitor renal medulla before, during and after ischaemia-reperfusion. The right renal artery was surgically exposed and fitted with an adjustable mechanical occluder. Renal medullary ^{1}H-NMR spectra showed that during 30 min ischaemia, lactate levels increased whereas sorbitol and TMAs decreased. After reperfusion sorbitol and TMA levels increased within 15–30 min,

and lactate decreased to pre-ischaemic levels over 1–2 hours. This technique appears to be suitable for differentiation of *in vivo* biochemical information between the renal cortex and medulla, and for assessment of *in vivo* renal ischaemia-reperfusion injury.

Mechanism of tubular uptake of filtered iron in rat remnant kidney (RK). *B.J. Nankivell, Y.C. Tay, R.A. Boadle, J. Chen, and D.C.H. Harris, Dept. Renal Medicine and EM Unit, Westmead Hospital, NSW 2145.* Iron accumulates within proximal tubular lysosomes in human and experimental chronic renal failure. The mechanism of accumulation has not been defined. The effect of iron-transferrin dissociation on tubular iron uptake was determined in RK ($N = 35$) and sham-operated controls (SO, $N = 16$) fed a $NaHCO_3$- or NaCl-supplemented diet for 8 wks. NaCl-fed RK rats had increased systolic blood pressure, final serum creatinine and decreased urinary pH. The substantial accumulation of iron, as demonstrated by energy dispersive X-ray analysis (EDS) in RK ($P < 0.01$ vs. SO), was not influenced by urinary pH, suggesting that dissociation of iron from transferrin did not occur in the acidic tubular-lumen. The filtered protein marker, horse-radish peroxidase (HRP, 6.25 mg/100 g BW), was also administered to RK and controls. Uptake of protein and iron by individual tubules was determined by EM histochemistry and EDS, on tissue fixed 15 and 60 minutes after HRP injection. Iron was present with HRP in apical lysosomes. After 15 minutes, HRP uptake correlated with numbers of iron-containing lysosomes ($r = 0.65$, $P < 0.01$). At one hour, secondary lysosomes (HRP-negative) contained less iron than recently reabsorbed, HRP-positive lysosomes ($P < 0.01$), consistent with release of lysosomal iron. Electron-dense focal accumulations of iron were demonstrated in tubular cytoplasm (but not nucleus) at high-magnification, by EDS. These data demonstrate in RK that filtered iron-transferrin can be reabsorbed intact across the brush-border membrane into tubular lysosomes along with bulk protein uptake, followed by release of iron and overload of cytoplasmic stores.

Effect of cyclosporin on macrophage infiltration and cellular proliferation in the Thy-1.1 model. *Mary Polihronis, Brendan F. Murphy, and David A. Power, Dept. Nephrology and Clinical Immunology, St. Vincent's Hospital, VIC, 3065.* Accumulation of glomerular extracellular matrix and proliferation of mesangial cells are important features of glomerulonephritis (GN) that progresses to end-stage renal disease. A model of mesangial proliferative GN can be induced with an antibody directed to the Thy-1.1 antigen on mesangial cells. Chemokines such as monocyte chemotactic protein-1 (MCP-1) and cytokines including platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) appear to be important in the pathogenesis of this model. In this study, therefore, we attempted to modify the Thy 1.1 model with Cyclosporin A, a cytokine inhibitor. Glomerulonephritis was induced in two groups of rats by a single intravenous injection of anti-thymocyte serum. One group of animals was treated with CsA 10 mg/kg daily, the other served as a control. The rats were sacrificed at various time points. Histologic examination of kidneys biopsies revealed significant differences between the control and CsA treated rats in the extent of macrophage infiltration and mesangial cell proliferation. At day 3 there was a significantly ($P < 0.01$) greater influx of ED-1 positive cells (6.47 $\pm$ 0.61) in untreated rats compared with CsA-treated animals (2.33 $\pm$ 0.61). Expression of α-smooth muscle actin, which has been associated with mesangial cell proliferation in this model, was also reduced in rats treated with CsA but there was no change in the number of cells expressing PCNA, a marker of proliferating cells. These results suggest that cyclosporin reduces the signals leading to macrophage infiltration within the mesangium in the Thy 1.1 model. Secondly, there is no direct relationship between mesangial cell proliferation and α-smooth muscle expression, as has previously been reported.

Renal hypertrophy in two rat models of cirrhosis. *M.A. Lonergan, S. Aglibut, and M.J. Field, Department of Medicine, University of Sydney, Concord Hospital, NSW 2139, Australia.* Avid proximal tubular sodium retention occurs in cirrhosis. Renal hypertrophy occurs in other circumstances of increased tubular transport. During studies of possible mediators of the altered sodium and water homeostasis that occurs in cirrhosis, we measured rat kidney weights to examine if alterations in renal mass were seen in these models. Two models of cirrhosis were utilised, that of carbon tetrachloride-induced cirrhosis (CCl_4), and bile duct ligation and resection (CBDL). Controls for the CCl_4 model consisted of rats receiving phenobarbitone alone and for the CBDL model, sham operated rats. Clearance studies were performed under Inactin anaesthesia after 16 weeks for CCl_4 and 4–5 weeks for CBDL, following which animals were bled for biochemical studies. Following exsanguination, the capsule of each kidney was stripped and the renal pedicle cut flush with the hilum. The kidneys were patted dry and weighed. In both models, the kidney weights, both absolute and relative (per 100 g of bodyweight) were significantly greater in the cirrhotic rats than in the controls ($P < 0.001$). In the CCl_4 model: the absolute weight of the right kidney of the Control rats ($N = 29$) was 1.48 $\pm$ 0.03 and of the CCl_4 ($N = 34$) 1.66 $\pm$ 0.04 grams; relative weight of the Control was 0.306 $\pm$ 0.004 and CCl_4 0.409 $\pm$ 0.013. In the CBDL model: the absolute weight of the right kidney of the Sham rats ($N = 20$) was 1.38 $\pm$ 0.03 and of the CBDL ($N = 27$) 1.52 $\pm$ 0.03; relative weight of the Shams was 0.33 $\pm$ 0.009 and of the CBDL 0.46 $\pm$ 0.013. The increased weight of the kidneys is consistent with renal hypertrophy occurring in response to the avid proximal tubular sodium reabsorption that occurs in cirrhosis. Although the changes in renal function that occur in cirrhosis are due primarily to hormonal effects resulting from the altered haemodynamics in this condition, the present results suggest that there are also intrinsic (structural) changes induced in the kidney that may contribute further to the altered tubular handling of salt and water. These findings justify further studies into the morphological basis of the hypertrophy, and into the possible mediating factors, such as hepatocyte growth factor.

Mast cells (MC) and type VIII collagen in human diabetic nephropathy (DN). *B. Rüger, R. Dunbar, Q. Hasan, N. Greenhill, H. Sawada, and T.J. Neale, Dept. of Medicine, Wellington School of Medicine, and Yokohama City University, School of Medicine, Yokohama, Japan.* Human MC are increasingly associated with angiogenesis, chronic inflammation and fibrosis but are not known to produce extra cellular matrix (ECM) components. Type VIII collagen is a non-fibrillar short chain collagen (also associated with angiogenesis) that forms a molecular bridge connecting ECM components. MC were identified in a number of tissues with monoclonal antibodies specific for human tryptase, chymase, and *c-kit* and histochemically with berberine sulphate, safranin and alcian blue. Many MC expressed type VIII collagen by immunohistochemistry using antibody specific for the α-1 chain. MC did not express the interstitial collagens I, III and V and pretreatment with collagenase abrogated type VIII collagen staining but did not prevent staining for tryptase. We examined the contribution of MC and type VIII collagen to the fibrosis occurring in DN. MC were prominent in the interstitium in DN ($N = 20$) and were located particularly perivascularly. Periglomerular fibres in DN stained intensely for type VIII collagen. There was a correlation between the proportion of MC^{TC} cells and the percentage MC positive for type VIII collagen in DN. RT-PCR identified the mRNA for type VIII collagen in kidney tissue. Digoxigenin-labelled anti-sense probes specific for type VIII collagen mRNA proved by *in situ* hybridisation that MC in the kidney produce type VIII collagen. Sense probe and RNase pretreatment controls were negative indicating specificity. MC proliferation and type VIII collagen production *in situ* may be early events in fibrosis, that are later assisted or completed by fibroblasts and their interstitial fibrillar collagen products. Our finding that type VIII collagen is produced by human MC in fibrotic tissues, particularly DN, provides the first evidence of a synthetic role for MC in the construction of connective tissue *in vivo*.

Collagen type III glomerulopathy: Extension beyond the glomerulus. *J.P. Dowling, I.K. Forbes, and S.T. Chou, Departments of Anatomical Pathology and Nephrology, Royal Melbourne Hospital, Parkville 3050, Victoria, and Pathology Repatriation Hospital, Heidelberg.* An unusual form of renal disease characterized by the mesangial, glomerular capillary loop and, perhaps, tubular basement membrane accumulation of material shown by immunohistochemistry to consist mostly of collagen type III has been increasingly recognized in both adults and children. The ultrastructural features are similar to those of the nail-patella syndrome (N-PS). We present a recent example of this rare lesion which developed in a 51 year old female of mid-European origin who for several years prior to the development of microscopic haematuria and mild proteinuria (0.6 gm/day) had been treated for hypertension, hypercholesterolaemia and peripheral vascular disease involving carotid and leg vessels. An intravenous pyelogram was normal. Antinuclear antibody titre was 1:150 and erythrocyte sedimentation rate 70 mm/hr. Serum creatinine at the time of renal biopsy was 0.10 mMol/l and she was receiving Enalapril. Renal

biopsy revealed striking mesangial acellular nodular expansion and capillary wall thickening with mild glomerular hypercellularity and mild tubulointerstitial damage with patchy thickening of tubular basement membranes, all due to the accumulation of lightly eosinohilic amorphous material. Immunohistochemistry showed irregular light to moderate IgM staining of capillary loops and mesangia and the amorphous material stained strongly in all areas with an anti-collagen type III antibody on streptavidin-biotin immunohistochemistry. Severe intracranial disease and cardiac failure with cardiac arrythmias developed and the patient died 20 months after renal biopsy. Autopsy findings revealed little if any progression in renal deposition of collagen III, atherosclerosis of aorta with involvement of coronary, carotid and renal ostia and evidence of myocardial and cerebral ischaemic damage. The renal lesion which may be part of a generalized disturbance of collagen metabolism appears distinct from NPS and is suggested by Gubler et al to have autosomal recessive as opposed to the autosomal dominant inheritance in N-PS. This is the first report to emphasize involvement of tubulointerstitial tissues in the process.

1. GUBLER MC ET AL.: *Pediatr. Nephrol.* 1993, 7:354–360

Modulation of cytokine and fibronectin expression in an animal model of focal glomerulosclerosis by a low protein diet. *Brigitte Schiller and John Moran, Dept. of Nephrology, Rush Presbyterian Medical Center, Chicago, IL, USA; Renal Division, Baxter Healthcare, McGaw Park, IL, USA.* We investigated cytokine expression in the puromycin aminonucleoside (PAN) model of focal glomerulosclerosis (FGS) in two groups of rats fed either a 24% (normal) or 6% (low) protein diet followed for 28 days. Cytokine expression was evaluated by Northern blot of pooled RNA from whole kidneys (3–5/group) hybridized with IL-1β, TNF-α, TGF-β, MCP-1 and fibronectin. Both PAN groups showed significant proteinuria from day 5, with a peak on day 10: (Mean ± SD) 679 ± 37 (24%) vs. 417 ± 63 mg/24 hours (6%). FGS was significantly less in the 6% group (7.5 ± 1.2 vs. 20.7 ± 3% of glomeruli, $P < 0.05$). TGF-β expression was found to be only slightly altered within the first 7 days in both PAN groups. However on day 10 a marked increase in both groups (2-fold in 6% group, 3-fold in 24% group) was found, which progressively increased until day 28 (5-fold in 24% group, 2.5-fold in 6% group). MCP-1 showed in the 6% group an immediate 6-fold increase on day 5, whereas the 24% results remained similar to control values. On day 28 we found a 3-fold increase in the 6% protein group, which was again lower in the 24% group at this time (2-fold). IL-1β or TNF-α expression was not detected. Fibronectin expression was more than 6-fold increased on day 28 in the PAN 24% group, whereas the 6% group was close to control levels. Conclusion: A low protein diet modulates the expression of MCP-1, TGF-β and fibronectin in kidneys developing glomerulosclerosis after PAN injection. This altered cytokine expression may explain the decreased FGS in rats on the low protein diet.

Focal glomerulosclerosis in the remnant kidney model: An inflammatory disease mediated by cytokines. *Brigitte Schiller, Michael DeLeo, Emily Warren, and John Moran, Dept. of Nephrology, Rush Presbyterian Medical Center, Chicago, IL, USA; Renal Division, Baxter Healthcare, McGaw Park, IL, USA.* We investigated cytokine expression in 5/6 nephrectomized rats fed a normal (24%) or low (6%) protein diet and compared to sham operated rats on the same diets. The rats on 6% protein had less proteinuria than the rats on 24% (Mean ± SEM: 2 weeks, 45.1 ± 1.07 vs. 126.7 ± 3.53; 4 weeks, 36.9 ± 4.51 vs. 175.8 ± 34.41 mg/24 h) and significantly less focal glomerulosclerosis (FGS) (17.4 ± 4.4 vs. 27.4 ± 8.8% of glomeruli at 7 weeks). Cytokine expression was evaluated by Northern blot of total pooled RNA extracted from whole kidneys (N = 3–5). Monocyte chemoattractant protein (MCP-1) was increased in both groups after 2 weeks to 3 times control levels. However, after 4 weeks MCP-1 expression decreased in rats on 6% protein (1.5 times control) and reached control levels after 7 weeks. In contrast, in the 24% group it increased further after 4 weeks (5.6 times control) and was still twofold higher after 7 weeks. Transforming growth factor-β (TGF-β) was found to be modestly upregulated in both groups at 2, 4 and 7 weeks to between 120 and 160%. IL-1β and TNF-α were not detected. Conclusion: In this model an initial "mechanical" injury results in a sustained inflammatory response within the kidney. MCP-1 and TGF-β may play a role in the pathogenesis of FGS in this model. A low protein diet modulates the expression of MCP-1 and improves the morphological outcome after renal ablation, suggesting an alternative mechanism to the "hyperfiltration" hypothesis.

Evidence for pre-eclampsia in a baboon pregnancy with twins. *A. Hennessy, A.G. Gillin, D.M. Painter, P.J. Kirwin, and J.S. Horvath, Departments of Renal Medicine and Anatomical Pathology, Royal Prince Alfred Hospital, Camperdown, 2050.* Preeclampsia (PE) is a syndrome of hypertension, oedema and proteinuria in pregnancy accompanied by hepatic damage if severe, which resolves after delivery. A pathognomonic renal lesion characterised by endothelial swelling, mesangial expansion, double contouring of the basement membrane and fibrin deposits has been described. This condition is almost exclusively a human disease and risk factors for its development include molar pregnancy, multiple pregnancy and primiparity. Primates rarely develop PE spontaneously. This paper describes the renal biopsy findings in a case of twinning in a baboon (*Papio hamadryas*) that had an otherwise symptomatically normal pregnancy. The aim of this study was to demonstrate the similarity of pathological changes in primate PE with those seen in human pregnancy. Methods. The animal had a twin pregnancy, duration 180 days (RR 182 ± 5 days). At 173 days an open wedge renal biopsy was performed and then repeated at five months post-partum (PP). This animal was studied as part of a wider study to examine the effect of uteroplacental ischaemia on renal morphology. Morphometry of the glomerular volume (light microscopy), basement membrane thickness (BMT) and mesangium volume (MV) on electron-microscopy (EM) were taken according to methods described previously. Results. The animal (anaesthetised) had a mean arterial pressure (MAP) of 104 mm Hg (RR 82 ± 7 mm Hg) at term and PP 88 mm Hg. There was a 200 fold increase in urinary protein excretion from 0.0014 mmol/gCr excreted at 139 days to 0.288 mmol/gCr excreted at term, with a decrease PP to 0.026. There was a decrease in platelet count from 365 × 10^9/l at 139 days to 245 × 10^9/l at term. PP the platelet count was 455 × 10^9/l. There was no other clinical or biochemical evidence of PE. The term biopsy demonstrated the presence of deposits, mesangial expansion and diffuse double-contouring. There were arterial as well as glomerular deposits seen on LM. These changes had resolved on the PP biopsy. A mild interstitial nephritis at the time of the term biopsy resolved. On EM there were fibrin deposits in the term biopsy which had resolved PP. Morphometry showed that MV and BMT were measurably decreased on the PP biopsy. Conclusions. Twinning in primates can be associated with renal morphological changes that resemble the changes seen in baboons with uteroplacental ischaemia and in humans with PE. The baboon remains a suitable and available model for studying the pathogenesis of preeclampsia.

ANA, anti-cardiolipin and anti-glomerular basement membrane antibodies in ANCA-associated diseases. *Judy Savige, Linus Chang, Russell Buchanan, D. Wilson, and D. Davies, University Department of Medicine, Austin Hospital, Box Hill Hospital, VIC and South Western Pathology Service, Liverpool, NSW.* A number of autoantibodies have been described in the ANCA-associated diseases. We have looked at the incidence of ANA, anti-cardiolipin (ACL) and anti-glomerular basement membrane (GBM) antibodies in serum from patients with Wegener's granulomatosis (WG), microscopic polyarteritis (MPA), segmental necrotising glomerulonephritis (SNGN) and ANCA-associated vasculitides (VASC).

	ANCA	ANA	ACL	anti-GBM
WG	30/36	7/36	10/25	2/25
MPA	30/34	16/34	8/14	1/14
SNGN	8/11	6/11	—	—
VASC	18/18	8/18	6/14	0/14

ANA were speckled (N = 23), homogeneous (N = 10) or nucleolar (N = 4). There was no correlation between titres of ANCA and ANA, and one antibody could disappear before the other. ANA were found more often in MPA than in WG and were associated significantly more often with pANCA than with cANCA ($P < 0.05$). Anti-cardiolipin antibodies were also common in the ANCA-associated vasculitides but there was no significant association with any disease type. Finally, anti-GBM antibodies occur in these diseases but are uncommon.

Detection of glucose-6-phosphate (G-6-P) derived advanced glycosylation end products in diabetic nephropathy. *B.G. McWilliam, B.M. Rüger, T.J. Neale, and R.P. Murray-McIntosh, Department of Medicine, Wellington School of Medicine, P.O. Box 7343, Wellington, N.Z.* Proteins may be nonenzymatically glycosylated eventually forming cross-linked, stable molecules termed "advanced glycosylation end products" (AGEps) which occur in humans with normal aging and at an accelerated rate in diabetes. Their accumulation is thought to be a biochemical link between chronic hyperglycaemia and the pathophysiological complications of diabetes, such as diabetic nephropathy. Glucose is the major circulating sugar and glucose-derived AGEps have been shown to accumulate in subjects with diabetes and/or renal impairment. We have detected abundant deposition of G-6-P derived AGEps immunohistochemically in renal biopsies exhibiting diabetic nephropathy and are therefore seeking to determine the involvement of these compounds in the disease. A competitive ELISA was developed specifically to detect these products utilising antibody against *in vitro* prepared G-6-P-derived AGE-keyhole limpet haemocyanin. *In vitro* prepared, G-6-P derived AGE-bovine serum albumin (AGE-BSA), (1 mM AGE-BSA = 11.55 A_{350}) was bound as the solid phase at a concentration of 5 ng/well. Whole blood was collected into heparinized tubes from four groups of subjects: those with diabetes mellitus with and without renal impairment (serum creatinine > 112 μMol/l), and nondiabetic patients with and without renal dysfunction. Erythrocytes were haemolysed and delipidated and insoluble material removed. The resulting haemolysate was assayed for AGEp content by competitive ELISA and protein content by the BCA reaction. The average AGE unit/mg protein in the haemolysate of patients who had renal failure was significantly higher (mean = 3.97 ± 2.91, $P = 0.0001$; and mean = 6.52 ± 9.11, $P = 0.0003$ for diabetic and non-diabetic patients respectively) than diabetic patients who did not have renal impairment (mean = 0.22 ± 0.13, $P = 0.7442$) compared to the control group (mean = 0.26 ± 0.16). These data show that glucose-6-phosphate derived AGEps accumulate in the circulation of subjects with renal impairment from any cause. Renal deposition of AGEps in diabetic patients may be the result of clearance of the increased amounts of AGEps formed during hyperglycaemia, or local formation *in situ.*

Isolation and characterisation of potentially bioactive advanced glycosylation end products (AGEPs) by lactoferrin (Lf) binding. *C.M. Brimer, R.P. Murray-McIntosh, and T.J. Neale, Department of Medicine, Wellington School of Medicine, PO Box 7343, Wellington South, New Zealand.* Circulating and extracellular matrix proteins have been shown to be nonenzymatically glycosylated *in vivo* and *in vitro* leading to the formation of AGEPs as brown and/or fluorescent moieties mainly on lysine residues. AGEPs have been implicated in the chronic complications of diabetes by alteration of protein character and in the inflammatory responses which they elicit. We have raised antiserum to *in vitro* prepared AGEPs and have detected, in patients with renal failure, enhanced binding in haemolysates by ELISA and staining of human diabetic kidney by immunohistochemistry. However only some of the epitopes present in the immunogen may be important in stimulating inflammatory or other responses in cells. The specific cell receptor which binds AGEPs contains a protein with N-terminal sequence identical to that of Lf, and antibody to Lf binds to this Lf-like binding protein. Further, AGEP-albumin binds to immobilised Lf. We have utilised this property of Lf to develop a competitive binding assay for those AGEPs that bind to Lf. A model AGEP, n-acetyl lysine methyl ester (ALME) incubated with glucose-6-phosphate (G6P), competes for Lf in this assay. Competition increased with time of sugar incubation, corresponding with increased brown and fluorescent character, and was greater than with ALME incubated with glucose:

	AGEP-Bovine Serum Albumin Equivalents (μg/ml) Mean (1 SD)			
	3 weeks	6 weeks	9 weeks	12 weeks
ALME+glucose	27.1 (4.7)	46.2 (5.1)	48.4 (5.2)	68.0 (6.1)
ALME+G6P	368.5 (34.9)	703.1 (59.5)	1115.9 (100.8)	1536.4 (153.6)

When separated on Biogel P10, high MW brown fractions (10 kD − >20 kD) competed for Lf, with no binding of lower MW fluorescent fractions. A Lf affinity column isolated and allowed further characterisation of potentially bioactive AGEPs from *in vitro* prepared mixtures. Activity of the column was confirmed by the binding of albumin incubated with G6P but not of control albumin. ALME incubated with G6P was applied and the bound fraction exhibited "brown" character. These Lf-binding AGEPs will be tested for bioactivity, used to study the nature of cellular responses, and to isolate antibodies with specificity for bioactive AGEP moieties in diabetic kidney and other tissues.

The humoral immune response in the lpr mouse. *K.G.C. Smith, D.M. Tarlinton, and G.J.V. Nossal, Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria, 3050.* The lpr mouse has a mutation in the fas gene and spontaneously develops an autoimmune disease similar to SLE. Fas is a cell surface molecule which can mediate apoptosis. It is expressed on activated T and B lymphocytes and in germinal centres. An intrinsic B cell defect explains part of the lpr phenotype. These findings suggest a role for fas in the humoral response to antigen. We investigated this by comparing the response to the hapten NP in wild type and lpr C57B1/6 mice. Changes in splenic cellularity after immunisation were similar in both groups. Total anti-NP IgM and IgG_1 and high affinity anti-NP IgG_1 rose to the same levels in both groups. NP-specific memory B cells were enumerated using 6 parameter FACS analysis at day 49 after immunisation and the findings confirmed by clonal culture of splenocytes. They made up 0.01% of splenocytes in both groups of mice. Finally somatic hypermutation was studied by sequencing V_H genes of NP specific cells. In summary splenic involution, affinity maturation of antibody and memory cell generation are all essentially unaffected by the absence of fas. Thus if fas-mediated apoptosis does play a role in humoral responses to antigen it is not critical and other mechanisms can compensate for its absence.

Polyarteritis overlap syndrome: A report of 7 cases. *G.S. Kirkland, J. Savige, P.J. Miach, W. Heale, R.N. Hope, and Roger Sinclair, Renal and Medical Units, University Departments of Medicine and Pathology, Austin Hospital, Heidelberg, Victoria, Australia 3084.* The diagnosis of Polyarteritis overlap syndrome with features of both microscopic polyarteritis (mPAN) and classical polyarteritis nodosa (medium vessel arteritis, cPAN) was made in 7 individuals. mPAN was demonstrated by renal biopsy ($N = 6$) or by heavy glomerular haematuria (1); cPAN was demonstrated by renal biopsy ($N = 3$) or histological examination of the small bowel (4). There were 5 males and 2 females with a median age of 61 years (range 60 to 77). Major clinical features were fever ($N = 6$), night sweats (5), gastrointestinal bleeding (4), proximal myopathy (3) and peripheral neuropathy (3). The serum creatinine at presentation ranged from 0.166 to 0.811 mmol/l. Investigations demonstrated a raised ESR (6), anaemia (6), thrombocytosis (6), hypoalbuminemia (6) and abnormal liver function tests (6). Five out of 5 individuals tested were positive for ANCA (2 cANCA, 2 pANCA and 1 not described). One patient died at admission from gastro-intestinal blood loss but the other 6 were treated with pulse and oral prednisolone, and cyclophosphamide. All 6 remain alive at follow-up after a median of 33 months (range 3 months to 8 years), and all but one have a serum creatinine below 0.2 mmol/l (range 0.106 to 0.209). Polyarteritis overlap syndrome is a not uncommon condition.

Epitope mapping of anti-proteinase 3 and anti-myeloperoxidase antibodies in ANCA-associated diseases. *Linus Chang and Judy Savige, University Department of Medicine, Austin Hospital, Heidelberg, Victoria.* The protein targets of antibodies may be either linear sequences of amino acids or conformational arrangements. Where the target is a linear sequence, this peptide could be used in a diagnostic assay; in addition any homology with bacterial or viral proteins would suggest that these agents might be important pathogenetically. The aims of this project were to determine whether anti-proteinase 3 and anti-myeloperoxidase antibodies recognise linear epitopes. We have used 3 techniques to look for linear epitopes: polyacrylamide gel electrophoresis (PAGE) under denaturing conditions, enzymatic digestion followed by separation on PAGE or by column chromatography, and an ELISA detection system using overlapping peptides (PIN method). We have shown that anti-proteinase 3 and anti-myeloperoxidase antibodies bind to the corresponding denatured reduced proteins in SDS in Western blots. However these antibodies do not appear to bind to small sequences after digestion with lysC. The antibodies do bind consistently to linear peptide sequences of the corresponding target protein but we have been unable to show that this binding is specific and the binding may reflect some properties of the peptides themselves. We conclude that linear epitopes are uncommon in the major targets of ANCA, proteinase 3 and myeloperoxidase.

Infiltration of the kidney by αβ and γδ T-cells: Effect on progression in IgA nephropathy. *M.C. Falk, G. Ng, G.Y. Zhang, G.C. Fanning, L.P. Roy, K.M. Bannister, A.C. Thomas, A.R. Clarkson, A.J. Woodroffe, and J.F. Knight, Renal Research Laboratories, The Children's Hospital, Camperdown NSW and Renal Unit, Royal Adelaide Hospital, Adelaide, SA, Australia.* The prognosis of IgA nephropathy (IgAN) is variable and difficult to predict. Previous studies have shown a link between lymphocytic infiltration in the interstitium and deterioration in renal function. We sought to confirm this finding and to further characterise the nature of the T-cells infiltrating the interstitium, based on the subtypes of T-cell antigen receptors (TCR), whether the abundant αβ TCR or the rarer non MHC linked γδ TCR, using reverse transcription (RT) PCR and immunohistochemistry. Thirty-six archival biopsies were obtained from the Royal Adelaide collection: 12 in each group of (a) non immunological renal disease (controls), (b) histologically proven IgAN with stable renal function for at least 12 months and (c) IgAN and progressive deterioration in renal function over at least 12 months. The biopsies had been snap frozen and stored at −70°C for up to 11 years. RNA was extracted, RT to cDNA and amplified using PCR with primers specific for β-actin, the constant gene segment of the alpha (Cα) and delta (Cδ) chains. The PCR products were dot blotted onto a nylon membrane and selectively probed with specific ^{32}P labelled probes. All specimens were studied in duplicate and the signal was quantified by two-dimensional densitometry. Results were expressed in arbitrary units as the ratio of TCR to β-actin signal for each biopsy. Biopsies with a poor densitometry score for β-actin were not included. Means were compared using the two sample T test. Where sufficient material was available, biopsies were also studied with monoclonal antibodies for CD3, TCRβ and TCRδ using peroxidase staining. Cellular infiltration was scored by a blinded observer on a four-point scale. Results (means for each group) are shown below:

	RT-PCR			Immunohistochemistry			
	n	C α:β-actin	C δ:β-actin	n	CD3	TCRβ	TCRδ
Progressive	7	41.84	28.60	9	2.89	2.67	0.78
Stable	7	19.12	6.27	7	1.29	1.14	0.17
Control	7	5.81	4.55	6	1.17	0.50	0.0

On RT-PCR significant differences were seen between controls and IgAN-progressive for Cα ($P < 0.01$) and for Cδ ($P < 0.01$). On immunohistology significant differences were seen for both types of T-cell between controls and IgAN-progressive (CD3 $P < 0.01$, TCRβ $P < 0.001$, TCRδ $P < 0.01$) and also between IgAN-stable and IgAN-progressive (CD3 $P < 0.01$, TCRβ $P < 0.01$, TCRδ $P < 0.05$). This study confirms the relationship between lymphocytic infiltrate and deterioration in renal function for patients with IgAN. αβ T-cells were increased in both stable and progressive disease. Significant numbers of γδ T-cells are only seen in biopsies of progressive IgAN. This new observation has prognostic and therapeutic implications.

Alteration in T lymphocyte function in children with nephrotic syndrome. *Stephen Alexander, Kevin Forsyth, and Ian K. Hewitt, Renal and Immunology Units, Princess Margaret Hospital, Perth, Western Australia.* To investigate the differences in lymphocyte response to immunosuppression in nephrotic syndrome, we studied suppression of lymphocyte proliferation in children with nephrotic syndrome. Ten patients were studied, 6 in remission off steroids, 3 who were steroid resistant and had required cyclophosphamide and 1 in relapse. There were 6 boys and 4 girls (ages 22 months–14 years 10 months). There were 10 controls in hospital for minor surgery. Five were boys and 5 girls (ages 27 months–13 years 5 months). Ten mls of blood was separated using ficoll-hypaque then washed with RPMI counted and suspended in RPMI with 10% FCS and 1% pyruvate, glutamine and non essential amino-acids. 10^5 cells were suspended in each well of a 96 well tray. Lymphocytes were cocultured with either PHA or anti-CD3 as mitogens and then either prednisolone or cyclosporin A were added in varying concentrations. The lymphocytes were incubated for 48 hours and pulsed for 18 hours with tritiated thymidine. Cells were harvested on a titretrek analysed on a scintilator and results expressed as a proportion of maximal response. Lymphocytes were also analysed using flow cytometry to study a range of subclass and activation markers. The lymphocytes showed a trend of delayed suppression in those stimulated by PHA but not in those stimulated by anti-CD3. There were no significant differences in the cell surface markers examined by flow cytometry.

Detection of mutations in Alport's syndrome. *C. Pham, Judy Savige, J. Agar, P. Miach, J. Dawborn, W. Heale, D. Wilson, and G. Thomas, University Department of Medicine and Renal Unit, Austin Hospital, Renal Unit Geelong Hospital and Department of Medicine, Box Hill Hospital, Victoria.* In most patients, the inheritance of Alport's syndrome is X-linked and in about 10% of these a mutation has been demonstrated in the alpha 5 chain of type IV collagen. We report here our results in a study of 9 affected families. DNA was extracted from peripheral blood leucocytes, cut with the restriction enzymes EcoRI, PstI and TaqI, electrophoresed, blotted and probed. The cDNA probes were derived from polymerase chain reaction (PCR) amplification of normal human mRNA using primers from the published sequence of the alpha 5 chain of type IV collagen. No novel alleles were demonstrated indicating that there were no large deletions nor sequence substitutions that altered the restriction enzyme recognition sites in any of these families. We suspected that autosomal recessive inheritance was implicated in one of these families and examined the DNA with cDNA probes from the alpha 3 and 4 chains of type IV collagen. Again however we found no mutations. We conclude that the majority of mutations in Alport's syndrome are single base pair substitutions, and some may affect a novel type IV collagen chain and non-coding regions of the gene. Finally we have shown that mRNA corresponding to the alpha 5 chain is present in both skin and bone marrow and these tissues can be used to look for single base pair substitutions using PCR and direct sequencing.

Assessment of adequate dialysis delivery in continuous ambulatory peritoneal dialysis (CAPD). *L.H. Fung, C.A. Pollock, L.S. Ibels, R.J. Caterson, D.A. Waugh, C. Macadam, and J.F. Mahony, Department of Renal Medicine, Royal North Shore Hospital, St Leonards, 2065, Australia.* Although adequate dialysis and its parameters are reasonably well established in conventional haemodialysis, its assessment in CAPD is as yet ill defined. The present study was designed to assess the relevance of urea kinetic modelling and the peritoneal equilibration test (PET) in CAPD in relation to nutritional markers and overall morbidity. Twenty-four hours dialysate and urine collections were made in 25 patients (13 M, 12 F; age 21–77 years) on CAPD for 15.2 ± 2.9 months, from which the Kt/V, protein catabolic rate (PCR), urea and creatinine clearances were determined. The following day patients underwent a standard four hours PET at which time serum albumin, cholesterol, triglycerides and total protein were also measured. Data regarding total body nitrogen (TBN) measurement within the preceding 6 months, hospital admissions and infectious complications within the last 12 months were reviewed. The study demonstrated that total Kt/V delivered was highly dependent on residual renal function ($P < 0.0001$), and Kt/V derived from peritoneal clearance diminished with increasing age ($P < 0.01$). However, a higher delivered total Kt/V was associated with increased PCR ($P < 0.05$), which in turn was associated with an improved TBN ($P < 0.05$). The TBN further correlated with peritoneal glucose absorption ($P < 0.005$). Hospital admission rate decreased with improved PCR, although it failed to reach statistical significance ($P = 0.067$). Infectious complications further correlated with time on dialysis ($P < 0.005$) and with dialysis protein loss ($P < 0.05$), but not with serum albumin ($P = 0.31$). Although Kt/V determinations did not correlate with clearances determined by the PET, the dialysate/plasma creatinine ratio (D/P Cr) did correlate with PCR ($P < 0.05$) and with TBN ($P < 0.001$) but not with infectious complications nor hospitalization. The D/P Cr further correlated with peritoneal glucose absorption ($P < 0.0005$) and inversely correlated with ultra-filtration rate ($P < 0.05$). In general, serum albumin did not correlate with morbidity, nor other markers of dialysis adequacy. However, in female patients the rate of hospital admissions decreased with improved albumin level ($P < 0.05$). In conclusion, nutritional parameters of TBN and PCR correlate with outcome on CAPD. An integral relationship exists between nutritional status and dialysis delivery, which is best assessed by urea kinetic modelling. Although D/P Cr is a reasonable marker of nutritional status in CAPD patients, its value in assessing the adequacy of dialysis delivery is yet to be determined.

Total body nitrogen (TBN) as a prognostic marker in maintenance dialysis. *C.A. Pollock, L.S. Ibels, B.J. Allen, W. Ayass, R.J. Caterson, D.A. Waugh, C. Macadam, Y. Pennock, and J.F. Mahony, Department of Renal*

Medicine, Royal North Shore Hospital, NSW, 2065. It is increasingly appreciated that adequate dialysis is reflected in an adequate nutritional state and both are necessary to achieve an ultimately good prognosis. In order to assess long term nutritional adequacy, 151 patients (77 M, 74 F), aged 55.3 ± 1.3 yrs on maintenance dialysis (73 HD, 78 CAPD), underwent measurement of TBN by neutron activation analysis. Dietary history, anthropometrics and serum albumin were also measured. Sixty-eight patients were reassessed 22.6 ± 2.1 (6–72) months later. In cross sectional analyses anthropometric measurements and dietary intake remained stable over time in all patients. However, a significant fall in TBN occurred in the HD population with increasing time on dialysis ($P < 0.05$). In the prospective analyses, the fall in TBN in HD patients ($N = 36$), expressed as a % of normal nitrogen in the matched population (Nitrogen Index), did not reach significance (87.6 ± 0.2 vs. 85.3 ± 1.8%; $P = 0.16$), but in the CAPD patients ($N = 25$) a significant increase in TBN occurred (89.2 ± 3.3 vs. 95.2 ± 3.1%; $P < 0.05$). In 7 patients who transferred from CAPD to HD a fall in TBN was evident 96.6 ± 7.2 vs. 85.3 ± 2.2%; $P < 0.05$). TBN correlated with total caloric intake estimated from the dietary history ($P < 0.05$), but not with estimated protein intake. During follow up 38 patients died. These patients were older ($P < 0.05$). In the CAPD population they had been on dialysis for a greater length of time (34.3 ± 5.3 vs. 15.5 ± 2.6 months; $P < 0.05$), although this was not the case in the HD patients. In all dialysis patients, those who died had a lower TBN expressed both as g/kg lean body mass (31.0 ± 0.6 vs. 33.5 ± 0.4; $P < 0.005$) and as the nitrogen index (87.2 ± 2.1 vs. 92.8 ± 1.4%; $P < 0.05$). A lower serum albumin was associated with mortality in the CAPD patients (35.8 ± 0.6 vs. 31.6 ± 3.8 g/dl; $P < 0.05$), but this relationship was not observed in the HD subpopulation. Anthropometric measurements and dietary history were similar in those who died compared with survivors. We conclude that CAPD exerts a favourable influence on TBN and that anabolism is possible. No such effect was observed in the HD patients. In both populations TBN is inversely associated with increased mortality which is not predictable from measurements of anthropometry or serum albumin. Total caloric intake determined by dietary history is a better index of nutritional adequacy than is estimated protein intake.

Accuracy, patient tolerance and time-efficiency of volumetric versus manual ultrafiltration (UF) on haemodialysis (HD). *D. Perrett, S. Porter, F.S.D. Kan, A. Parnham, Incentre Dialysis Staff, G. Thatcher, P. Hurst, and M. Thomas, Dept. of Nephrology, Royal Perth Hospital, WA 6001.* To determine whether the added cost of volumetric UF devices affects the accuracy, tolerance or nurse-time efficiency of maintenance HD, a cross-over comparison with manual UF (via calculated trans-membrane pressure) was performed in 12 patients with recurrent hypotension on Incentre hospital HD. The mean absolute difference between the desired and actual post-dialysis body weight, the incidence of hypotension (fall in systolic BP > 25 mm Hg or need for saline), visual analogue symptom (VAS) scores (nausea, headache, cramp, itch and well-being) and stop-watch-recorded nurse UF calculation times were studied for 3 dialyses on each form of UF on 2 consecutive weeks. All patients used cuprophane membranes, Cobe Centry 2 HD machines, bicarbonate dialysate, with no change in dialysis prescription, dialysis nurse or clinical status during the study period. The sample size was selected to detect a difference in desired versus actual post-dialysis weight of 0.3 kg between the 2 forms of UF with 90% power at .01 level of significance. Data was expressed either as mean (SD) and categorically (improved, unchanged, worsened) and analysed by one-way ANOVA and Chi-square test. All patients completed the study. There were no significant differences between volumetric and manual UF in any parameter either pre-HD (not shown) or post-HD (Table), including nausea, headache and itch (not shown).

UF Mode	Weight diff *(kg)*	Systolic BP fall *(mm Hg)*	Well-being *(VAS, 0–10)*	Nurse-time *(secs)*
Manual	0.2 (0.1)	25 (17)	3.8 (3.0)	297 (106)
Volumetric	0.3 (0.1)	14 (17)	3.8 (3.1)	256 (118)

This study shows no justification for the use of volumetric UF in maintenance HD using cuprophane membranes.

The effect of controlled lipid lowering diet on plasma lipids of dialysis patients. *D. Saltissi, C. Morgan, and B. Knight, Department of Renal Medicine, Royal Brisbane and Keperra Hospitals, Brisbane, Queensland.* Dietary manipulation is advocated as primary therapy for dyslipidaemia. In Uraemia the complex requirements/restrictions make adherence to NHF lipid lowering dietary guidelines and patient compliance difficult. Also there is no data to support benefit of such manipulation. We have conducted a 14 week study of dietary control, in dialysis patients, limiting total fat intake to 30% (PD) and 40% (HD) of total calories with as close a 1:1:1 poly:mono:saturated fat ratio as possible. Results:

Mean values: standard errors omitted for brevity

	TC		Trig		HDL		LDL		VLDL	
Weeks	0	14	0	14	0	14	0	14	0	14
HD + 32 pts	5.70	5.65	2.40	2.10	1.02	1.06	3.70	3.60	0.95	0.97
	P = NS		P = NS		P = NS		P = NS		P = NS	
PD 10 pts	6.33	6.13	2.14	2.36	1.19	1.02	4.15	3.41	0.97	0.98
	P = NS		P = NS		P = 0.0225*		P = NSP		P = NS	

42 pts (12 M, 30 F; age 38–75, mean 59.4 yrs)
* Associated with fall in monounsaturated fat
\+ Associated with a fall in total energy

Further data will be presented. Current dialysis diets are close to optimum. Further manipulation according to NHF lipid lowering recommendations may be deleterious. Treatment of uraemic dyslipidaemia is likely to require pharmacological therapy.

A new, non-invasive method of measuring blood volume (BV) during haemodialysis using optical techniques. *D.W. Johnson, M.P. McMahon, S.B. Campbell, and S.J. Fleming, Department of Renal Medicine, Royal Brisbane Hospital, Qld, 4029.* To aid in the clinical assessment of patient hydration state, we developed an inexpensive method of measuring BV during haemodialysis, based on the linear relationship between BV and haemoglobin concentration ([Hb]) and between [Hb] and near-infrared light absorption by blood. An optical monitor and light source were clamped across transparent blood lines in 10 haemodialysis patients in order to provide a continuous recording of [Hb]. In each patient, a strong linear correlation was found between received optical power and [Hb] concentration ($r \geq 0.975$). By analysing the rates of change of [Hb], and therefore of BV, at two different rates of fluid removal and solving 2 simultaneous equations, BV was determined in 10 patients and compared with the concomitant BV measured by the standard technique of radio-isotope dilution. Using linear regression, a statistically significant, strong correlation was found between the two methods ($P = 0.004$, $r = 0.822$, $y = 3.707 + 0.225x$). The overall accuracies of the spectrophotometrically-derived BV measurements were of the order of ± 400 ml. The advantages of the technique included its simplicity, low cost, rapidity, repeatability, safety and portability. In conclusion, we found that this practical new technique could accurately measure BV during a haemodialysis session, thus providing a useful adjunct to the clinical assessment of hydration state.

Calcium acetate (CA) versus calcium carbonate (CC) in haemodialysis patients. *J. Connolly, M. Mantha, and D.C.H. Harris, Department of Renal Medicine, Westmead Hospital, NSW 2145.* Although available overseas for several years, CA has only just become available in Australia. To test a new Australian formulation CA was compared to CC in a 24 week randomised, blinded, cross-over study in 31 maintenance haemodialysis (HD) patients, aged 51 ± 24 (SD) yrs. Data up until the time of crossover (12 weeks) are available. During and following an initial run-in period of 4 weeks 14 patients received CA first, and 17 patients CC. Mylanta was added when serum phosphate was > 1.80 mmol/L with maximum tolerated dose of CA or CC. Prior to crossover, serum calcium was 2.37 ± 0.15 (CA) and 2.46 ± 0.21 (CC) mmol/L, P = NS. Serum phosphate was 1.36 ± 0.21 (CA) and 1.52 ± 0.21 (CC) mmol/L, P = NS. There were 2 episodes of hypercalcaemia (≥ 2.70 mmol/L) in 1 patient with CA, and 5 episodes in 2 with CC. Serum phosphate was ≥ 2.00 mmol/L on 2 occasions in 2 patients on CA, and on 5 occasions in 2 on CC. CA dose at crossover was 2000 ± 912 mg Ca^{2+} day, and CC dose 2356 ± 2072 mg Ca^{2+}/day. Although mean Mylanta dose was similar in the both groups

(1000 mgs/day, 6 patients on CC required Mylanta, whereas only 2 on CA did). There were 5 dropouts during CA (due to renal transplant in 1, severe itching in 2, diarrhoea in 1, and 1 non compliance) and 5 during CC (2 died of unrelated cause, 3 non compliance). Preliminary results of this trial suggest that this formulation of CA is a satisfactory alternative to CC in HD. Compliance with CA may be improved by increasing tablet strength.

Tumour necrosis factor alpha (TNF-α) in human membranous nephropathy (MN): A role for glomerular epithelial cells (GEC). *T.J. Neale, H. Macaulay, B.M. Rüger, A. Bourke, R. Dunbar, and Q. Hasan, Dept. of Medicine, Wellington School of Medicine.* TNF-α, a pleiotropic and pro-inflammatory cytokine has been implicated in the pathogenesis of experimental glomerular disease. We examined its role in biopsy-proven human GN by monoclonal antibody immunohistochemistry on paraffin-embedded tissue ($N = 78$), digoxigenin-labelled oligonucleotide anti-sense probe *in situ* hybridisation, immunogold electron microscopy, quantitative immunoassay (ELISA and IRMA) ($N = 64$) and urinary immunoblot. Striking glomerular capillary wall and GEC staining was observed in all cases of MN ($N = 13$) and in membranous lupus nephropathy ($N = 3$ of 12). By immunogold EM TNF-α was localised to the GBM in MN especially in relation to immune deposits and within the GEC. Minimal change, FGS and IgA nephropathy sections showed no GEC or GBM TNF-α staining. *In situ* hybridisation localised TNF-α mRNA exclusively to GEC in all biopsies with membranous morphology but not in other histologic sub types. [TNF-α] was increased above normal in the serum and urine of patients with a variety of forms of GN but with statistical significance only in the urine of patients with MN (mean 23.2 ± 12 pg/ml, range 10–56, versus controls ($N = 20$): mean 5.32 ± 3.7, range 0–14 pg/ml; $P < 0.05$). Immunoblot of urine revealed monomer or trimer TNF-α in all patients with MN. Auto antibodies against TNF-α were detected in 14/64 GN patients; 9/14 were associated with elevated urinary [TNF-α]. The upregulation of TNF-α mRNA and expression of the protein in MN GEC and GBM further supports the contention that the GEC are targets for injury in MN. TNF-α may contribute from this source to glomerular capillary wall injury in MN resulting in proteinuria.

Epidermal growth factor (EGF) in remnant kidney model of glomerulosclerosis (GS): Effect of enalapril treatment. *A.M. Walker, and N.M. Thomson, Dept. Medicine, Monash Medical School, Alfred Hospital, Prahran, Melbourne, 3181, Australia.* We have recently reported alterations in EGF and its mRNA expression in rats developing GS following 7/8 nephrectomy. Enalapril has been demonstrated to inhibit compensatory renal growth (CRG) and slow the development of renal failure and glomerulosclerosis in rat remnant kidney model of chronic nonimmune injury. We have therefore studies the effect of enalapril on EGF and EGF mRNA expression in this model of GS. Twelve week old, male inbred SD underwent either 7/8 nephrectomy by infarction or sham laparotomy (Sh). Rats were randomly assigned to receive enalapril in drinking water (ENx) or water alone (Nx) and progression of renal failure followed. Animals were killed at regular intervals over a 12 week period and tissue assessed for histological damage. Tissue expression of EGF was determined by immunoperoxidase using rabbit anti-mouse EGF polyclonal antibody and EGF mRNA was quantitated by Northern blot analysis. When assessed at 12 weeks, enalapril had prevented the compensatory increase in renal weight (1.13 ± 0.12 g ENx, 1.88 ± 0.18 g Nx, 1.26 ± 0.06 g Sh), had reduced the rate of loss of renal function (s Cr 120 ± 18 mmol/l ENx, sCr 163 ± 8 Nx at 12 w) and had minimised proteinuria (95 ± 30 mg/day ENx, 241 ± 102 mg/day Nx at 12 w). Glomerular hypertrophy developed more slowly with enalapril treatment and glomerulosclerosis (GS) evident in only 6% glomeruli compared with 40% glom. in Nx group at 12 w. Preservation of tubulointerstium was observed in the treatment group. Epidermal growth factor protein was expressed in the distal convoluted tubules of all kidneys collected before the 3rd postoperative week in the Nx animals but had disappeared by the 6th week. Enalapril treatment (ENx) preserved the EGF expression at all time points examined. mRNA expression was increased at week 1, then progressively fell to levels comparable to sham by the 6th week. There was a subsequent increase in mRNA expression at later time points in the untreated animals. Thus there was a discrepancy between EGF expression and expression of EGF mRNA in animals developing GS. Whether this represents inhibition of EGF production or loss of the protein remains unresolved.

Clearance of a bacterial challenge to the peritoneal cavity in subjects undergoing peritoneal dialysis. *T.E. Miller and G. Findon, Department of Medicine, University of Auckland, Auckland, NZ.* Dialysis, and the associated dilution of host defenses in the peritoneal cavity, is thought to be an important factor contributing to peritonitis in CAPD. We have used an animal model of CAPD to explore the effect of dialysis on the clearance of several pathogens commonly introduced into the peritoneal cavity at the time of bag exchange. The experiments have shown that the ability of the host defense mechanisms to clear a bolus challenge remains largely intact despite considerable, dialysis associated changes to the peritoneal milieu. Substantial challenges of up to 10^8 viable microorganisms were either cleared from the peritoneal cavity or reduced to small but persistent numbers in the case of *S. aureus*, *S. epidermidis* and *Candida albicans*. *Ps. aeruginosa* on the other hand became established in the peritoneal cavity in large numbers after an initial reduction. Challenges with *S. aureus* and *Ps. aeruginosa* led to adhesion formation which encased the cannula and prevented dialysis. Clearance of viable organisms in the presence of implanted cannula and dialysis is summarized in the table.

	Cannula only	Cannula and dialysis	Adhesion formation
S. epidermidis	++++	+++	−
S. aureus	++++	+	+
Ps. aeruginosa	++	−	+
C. albicans	++	++++	−

We conclude that contrary to expectations, host defences in the peritoneal cavity appear intact in dialysed subjects challenged with pathogens commonly causing peritonitis in humans. Furthermore, adhesion and cannula blockage appear to be a microbial rather than host determinant.

Tissue factor pathway inhibitor is produced by rabbit renal cortical tissue. *J. Apostolopoulos, J.H. Erlich, S.R. Holdsworth, and P.G. Tipping, Department of Medicine, Monash University, Clayton, Vic 3168.* Tissue factor (TF) is the most important initiator of coagulation in inflammatory tissue injury. Glomerular expression of TF is markedly upregulated in forms of glomerulonephritis with prominent fibrin deposition. Tissue factor pathway inhibitor (TFPI), recently cloned for human and rabbit, is the major inhibitor of TF. TFPI is made predominantly by endothelial cells and has been shown to be upregulated by inflammatory cytokines (eg IL-1 and TNF-α) *in vitro*. However, its expression in renal tissue and its participation in glomerulonephritis (GN) has not been described. The aim of the current study was to a) determine whether TFPI is expressed in normal renal tissue b) to assess whether TFPI is regulated by proinflammatory signals and c) determine whether TFPI mRNA expression is altered during anti-GBM GN. Northern blot analysis using a cDNA probe to rabbit TFPI (a generous gift of Dr. James Wun) that hybridises to the two known TFPI mRNA species (4.0 and 1.4 Kb) was used to assess TFPI mRNA levels in a) normal rabbit cortex b) rabbits injected with LPS and c) rabbits up to 4 days post-induction of anti-GBM antibody initiated crescentic GN. TFPI mRNA was detectable in normal rabbit kidney cortex and TFPI mRNA and was upregulated in LPS treated rabbits. On day 1 of anti-GBM disease, glomerular inflammation was associated with macrophage influx, fibrin deposition and down-regulation of TFPI mRNA. TFPI mRNA levels returned to normal by day 4. This study thus demonstrates that TFPI is expressed in renal cortex and is rapidly down-regulated in crescentic anti-GBM GN with fibrin deposition.

T lymphocyte initiated glomerulonephritis requires P selectin interaction. *X.R. Huang, P.G. Tipping, M.C. Berndt, and S.R. Holdsworth, Monash University Dept. of Medicine, Monash Medical Centre, Clayton 3168 and Baker Medical Research Institute, Prahran.* Immediate injury in autologous phase anti-GBM antibody initiated GN has many features characteristic of delayed type hypersensitivity (DTH). Sensitized rats rechallenged with sheep globulin 5 days later (in the skin by subcutaneous injection and in the glomerulus by i.v. injection of sheep anti rat GBM globulin) develop delop a local T cell dependent macrophage influx and injury characteristic of DTH. The requirement for endothelial P selectin in T cell and macrophage localization was assessed by the administration of rabbit anti P Selectin antibody one hour before antigen rechallenge. Control rats given normal rabbit globulin developed immediate proteinuria (42.1 ±

14.1 mg/24 hrs mean ± SD normal 6.3 ± 3.2 mg/24 hr) together with T cell (1.0 ± 0.06 cells/glom cross section c/gcs; normal 0.1 ± 0.02 c/gcs) and macrophage (4.2 ± 0.26 c/gcs; normal 0.1 ± 0.1 c/gcs) infiltration. Dermal rechallenge results in skin swelling (sheep globulin response 3.1 ± 0.7 mm; horse globulin response 3.1 ± 0.7 mm ($P < 0.05$). Rats given anti-P selectin antibody had significantly less proteinuria (13.8 ± 7.3 mg/24 hrs) as well as glomerular T cell (0.3 ± 0.06 c/gcs) and macrophage influx (0.7 ± 0.13 c/gcs) all $P < 0.05$. Dermal skin response to sheep globulin rechallenge was significantly reduced to control levels (1.8 ± 0.1 mm $P < 0.05$). These results suggest that the primary effector response in dermal and glomerular DTH is P selectin dependent T cell recruitment.

Clusterin expression in experimental cyclosporine A (CyA) nephrotoxicity. *T. Hewitson, I.A. Darby, C. Jones, M. Fraenkel, and G. Becker, Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne and Department of Nephrology, Royal Melbourne Hospital, Victoria 3050 Australia.* *Objective:* Clusterin (rat sulphated glycoprotein-2) has been isolated from several tissues including the kidney and is thought to be induced during tissue damage. We have examined the expression of the clusterin gene in the kidney during the development of cyclosporine A (CyA) induced nephrotoxicity in the rat using *in situ* hybridization histochemistry. *Design and Methods:* Female Sprague Dawley rats (170 g) were divided into experimental or control groups and were given intraperitoneal injections of CyA (25 mg/kg) or vehicle respectively for 2, 4, and 6 weeks. Kidneys from animals sacrificed at these times were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were hybridized using either a sense or antisense riboprobe of 1100 bases complementary to clusterin mRNA which was labelled with P^{32}. *Results:* Clusterin gene expression was not detected in control animals. Expression of clusterin in distal convoluted tubules of CyA treated animals was evident at 2 weeks and increased substantially at 4 weeks and 6 weeks. The increase in clusterin positive tubules was associated with progression of tubulointerstitial nephritis. At 6 weeks clusterin expression was also seen in afferent arterioles, the glomerular capsule and transitional epithelium of the renal pelvis of CyA treated rat kidneys. No specific labelling was seen at any time with the sense probe. *Conclusions:* CyA nephrotoxicity is associated with a progressive increase in clusterin gene expression in tubules and arterioles, which precedes histopathological lesions and may be a marker of cell injury.